A RANDOMIZED TRIAL OF VITAMIN E AND SELENIUM: PLAUSIBILITY OF EFFECTS ON LUNG FUNCTION DECLINE

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Vitamin E and selenium are proposed to affect the oxidant-antioxidant balance in the body and thus to affect human health outcomes. The effects of these nutrients on rate of decline in lung function were assessed in the Respiratory Ancillary Study (RAS) to the Selenium and Vitamin E Cancer Prevention Trial (SELECT), a randomized placebo-controlled trial of men assigned to vitamin E (400 IU/day *all rac*-α-tocopheryl acetate) and/or selenium (200 μg/day L-selenomethionine). This dissertation reports the effects of supplementation on a biomarker of oxidative stress and on changes in plasma nutriture, and investigates the mediation and modification of the effect of supplementation on rate of decline in lung function.

Compared to placebo, vitamin E, alone and combined with selenium, lowered a biomarker of oxidative stress, urine F_2-Isoprostanes, by 21% (p=0.0231) and 9% (p=0.3738), respectively. Treatment with vitamin E attenuated the age-related decline in lung function, but this effect was not mediated by lowering of F_2-Isoprostanes. There was no evidence of selenium

effects on F_2-Isoprostanes (8% higher F2-IsoPs compared to placebo; p=0.5217).

Given no overall effect of selenium on rate of decline in lung function, a plausibility analysis considered whether treatment effects were limited to men with lower selenium status at study baseline. There was no difference in the effect of selenium on lung function by baseline plasma selenium concentration, either considering selenium deficiency, or plasma selenium as a continuous variable.

The effect of vitamin E on lung function is postulated to reflect effects in the lung compartment, and plasma vitamin E concentrations are assumed to reflect target tissue concentrations. We investigated factors associated with the change in plasma alpha-tocopherol in response to supplementation in the RAS. Race was the primary factor identified: specifically, the change in plasma alpha-tocopherol concentrations in response to alpha-tocopherol supplementation was about 50% less in African Americans compared to European Americans.

Vitamin E reduces systemic oxidative stress and attenuates age-related decline in lung function. Given differences in plasma response to supplementation, future studies considering baseline nutriture and indicators of nutrient absorption, transport and metabolism would contribute to a fuller understanding of vitamin E effects on health outcomes.

BIOGRAPHICAL SKETCH

Kristin Ann Guertin was born in Merrick, New York, where she resided with her parents and three younger sisters until moving to Ithaca, New York to attend Cornell University. At Cornell, Kristin majored in Biology & Society, earning a Bachelor of Science and graduating Cum Laude in 2005. Kristin was first exposed to epidemiology as an undergraduate in Dr. Pat Cassano's course in 2004, and it is then that she realized that she wanted to be an epidemiologist. She earned an MPH in Chronic Disease Epidemiology at Yale University in 2008, and then moved back to Ithaca in 2008, where she served as study coordinator for the Respiratory Ancillary Study (RAS) to SELECT, led by Dr. Pat Cassano. Motivated by the work of others in the Cassano research group, and under the mentorship of Dr. Pat Cassano, Kristin entered the graduate program in Nutritional Sciences as a PhD student in 2009. Kristin continued to serve as the RAS study coordinator alongside her work as a student, and from 2011-2012, Kristin held the position of study coordinator for a pilot study out of the Division of Nutritional Sciences, Engaging Health, Agriculture and Nutrition through the Cornell Experience (EnHANCE).

Kristin and her husband Michael, another Cornell alumnus, welcomed their son Evan in March 2012. In their free time, they enjoy sharing their love of the outdoors with Evan. Evan has offered endless entertainment and a welcome distraction to the process of writing a dissertation. As she prepares to leave Ithaca, Kristin looks forward to the many adventures ahead in both her academic and leisure activities. Cornell and Ithaca will always have a special meaning for Kristin, and she truly enjoyed her time in the Division of Nutritional Sciences both as an employee and student. Kristin plans for a career in research as a

chronic disease epidemiologist, utilizing the tools she honed during her time at Cornell and Yale. She is very grateful to have found a fulfilling career which offers the opportunity to ask novel, important questions which have the potential to impact public health.

To MJG and ELG

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CHAPTER 1

INTRODUCTION

Lung function measurements provide insights into lung health, which is predictive of mortality in adults, and are used to diagnose and stage the severity of chronic obstructive pulmonary disease (COPD). COPD, characterized by irreversible air flow limitation and inflammation, is the third leading cause of death in the United States and leads to morbidity, mortality, and contributes to increased health care costs (1). There is no cure for COPD, and although currently available therapies alleviate symptoms, no current treatments are efficacious in slowing the rate of decline in lung function(2-4), which is steeper in individuals with COPD. Smokers are at greatest risk of COPD and smoking cessation is the primary preventive approach that attenuates rate of lung function decline, however, cross-sectional lung function is lower in smokers even after cessation. Given that never and former smokers may also develop COPD, and given that about 20% of the US population persists in smoking, interventions that attenuate rate of decline in lung function are needed.

Observational epidemiological studies of dietary antioxidant intake, serum antioxidant concentration, and lung outcomes report that lower levels of antioxidant defense are associated with decreased lung function(5-11), supporting the hypothesis that oxidant-antioxidant balance is critical in maintaining lung health; thus, we hypothesized that supplemental antioxidants protect lung tissue from oxidative damage and attenuate rate of decline in lung function. Vitamin E and selenium, the two nutrients addressed

herein, are antioxidant nutrients hypothesized to affect lung function primarily through mechanisms related to the lowering of oxidative stress.

This dissertation research used data from the Respiratory Ancillary Study (RAS)(12), an ancillary study to The Selenium and Vitamin E Cancer Prevention Trial (SELECT)(13). SELECT began in 2001 and randomized 35,433 male participants to 400 mg α-tocopherol (vitamin E) + selenium (Se) placebo, vitamin E placebo + 200 μg Selenomethionine, 400 mg α-tocopherol+ 200 μg Selenomethionine, or vitamin E placebo + Se placebo; the primary endpoint for SELECT was the prevention of prostate cancer, whereas the primary endpoint for the RAS was rate of decline in pulmonary function. RAS was comprised of a subsample of 2,920 SELECT participants which was purposefully enriched with current cigarette smokers to test hypotheses in the subset of smokers; further details of RAS (NCT00241865) and SELECT (NCT00006392) are published elsewhere(12, 13).

Vitamin E is an essential fat-soluble vitamin that primarily functions as a chain-breaking antioxidant in humans, maintaining the integrity of cell membranes by eliminating free radicals(14) and thus preventing damage from reactive oxygen species (ROS). Vitamin E is comprised of four tocopherols and four tocotrineols which are distinguished by their chemical structures. Gamma(γ)-tocopherol is the main dietary form of vitamin E(14), however alpha(α)-tocopherol is the only form recognized to meet human requirements largely due to the preferential binding of this form by the α-tocopherol transfer protein (α-TTP) in the liver(15) and thus retention of this form in the plasma. Unless otherwise specified, vitamin E hereafter refers to α-tocopherol.

Selenium also has antioxidant properties and is an important constituent in protective enzymes and amino acids, such as selenocysteine and selenomethionine(16). Prior studies investigating the effects of selenium on health outcomes are mixed, but suggest that benefits may be limited to individuals with low baseline selenium status(17).

Previous randomized controlled trials of the effect of antioxidants on respiratory outcomes have produced mixed results(18-21), with most finding no effect of vitamin E on lung health outcomes. The lack of effects in past studies may be affected by misclassification of disease status, which would limit power to detect true associations. For example, SELECT used self-reported physician's diagnosis of COPD to define the COPD endpoint; self-reported diagnosis of COPD includes only a subset of individuals in the population with obstructive lung disease(22), and therefore studies that rely on self-reported outcomes are comprised of only a fraction of all outcomes. Previous work shows that patients with milder disease are less likely to be diagnosed(23), which may lead to biased estimates of preventive effects of intervention. Additionally, the duration of prior intervention studies may have been too short thus limiting the ability of the trial to demonstrate significant protective effects of vitamin E on COPD incidence, symptoms, and/or death or hospitalization from respiratory illness. If oxidative stress is truly reduced by vitamin E and such reduction alters disease risk, then studies of longer duration using more robust definitions of COPD (such as spirometry-defined COPD) may be needed in order to detect effects of interest. Of note, the one study reporting a significant finding of a protective effect of vitamin E had twice the duration (9.8 years)(18) in comparison to studies reporting no effect of intervention, which supports the idea that limited follow-up may have reduced the ability of prior studies to detect a significant effect of vitamin E on

lung outcomes. Lung function can be assessed by various lung function parameters including FEV_1 (forced expiratory volume in the first second of exhalation), and FEF_{25-75} (forced expiratory flow rate between 25-75% of the forced vital capacity (FVC)). Smokers have steeper rates of decline in FEV_1 and are at increased risk of COPD. Attenuating the rate of decline in lung function may have a significant public health impact, given that steeper rate of decline is independently associated with mortality(24-29) and with increased risk of COPD, but no currently available therapies (other than smoking cessation) attenuate the rate of decline(2-4). The RAS to SELECT previously established a protective effect of vitamin E (vitamin E alone or vitamin E combined with selenium) on rate of decline in lung function(12) rate of decline in FEV_1 was attenuated by 6 mL/yr for FEV_1 (p=0.188), and rate of decline in FEF_{25-75} was attenuated by 13mL/sec/yr (p=0.212); the latter effect was statistically significantly stronger in smokers ($p_{interaction}$=0.0513), the subgroup hypothesized to be at greatest risk of COPD and steep rate of decline. RAS found no effects of selenium on lung function endpoints.

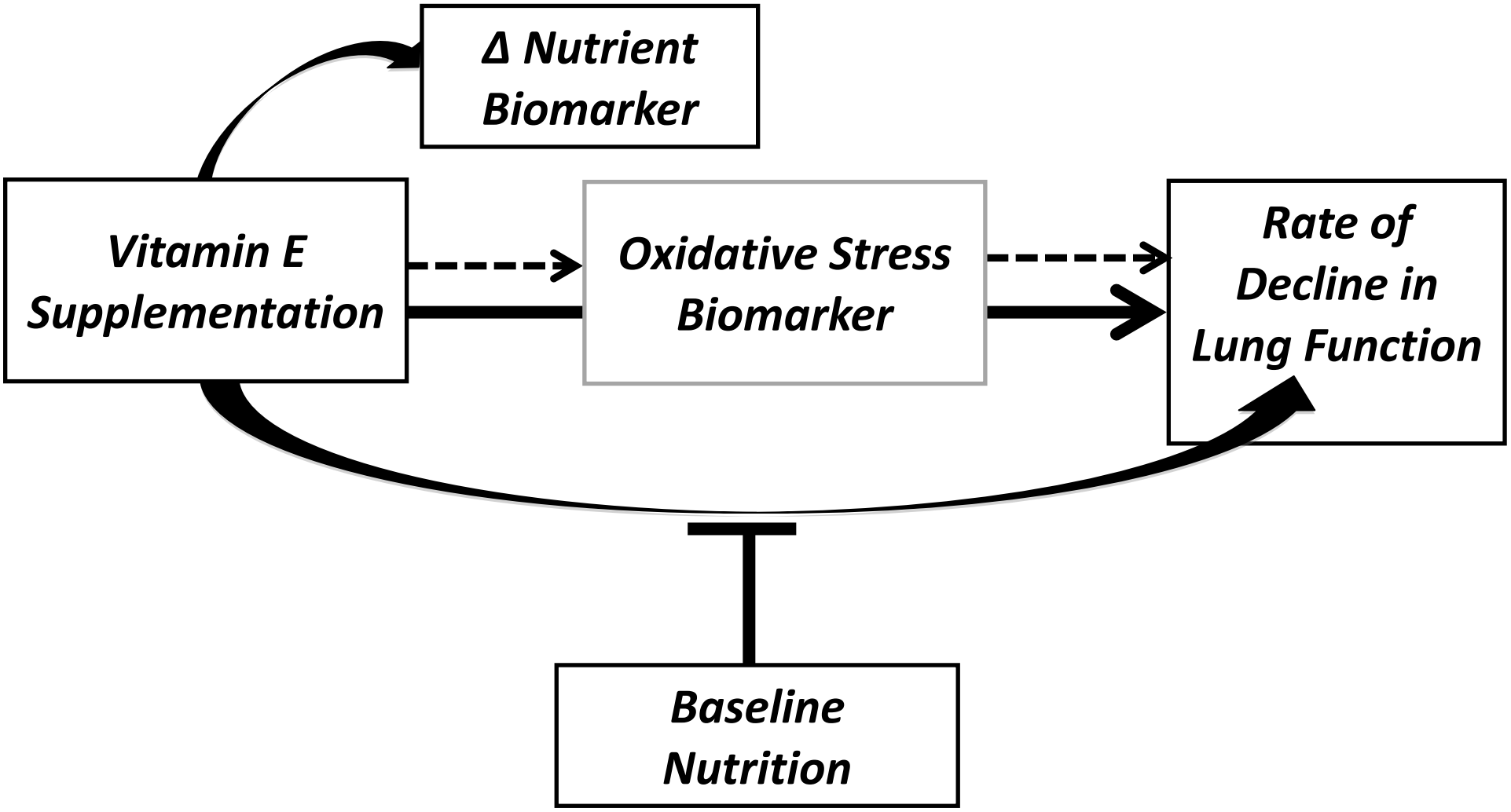
The epidemiologic framework for this dissertation research is presented in Figure 1. This research addresses the effects of vitamin E and selenium on the gold-standard biomarker of *in vivo* oxidative stress, namely urine F_2-isoprostanes (F2-IsoPs) (30), and further investigates whether the effect of vitamin E intervention on rate of decline in lung function is mediated by lowering of this biomarker. Past trials of antioxidant nutrients on biomarkers of oxidative stress and lung function have been limited by small sample sizes, short trial duration, cross-sectional and non-randomized designs, varying and sometimes unspecified nutrient forms and dosages, and ill-defined sample characteristics. This study improves upon past designs and offers more definitive evidence of the antioxidant effects

of vitamin E through utilization of a gold-standard biomarker of oxidative stress within a large, long-term randomized trial, and offers a more comprehensive investigation of the mechanism of action by investigating mediation of the effect of supplementation by F2-IsoPs.

Furthermore, this dissertation thoroughly considers all effects of these nutrients, including analyses of baseline levels of vitamin E and selenium. We hypothesized that individuals with lower baseline levels of a nutrient would have greater benefit from supplementation with that nutrient, and thus greater attenuation in rate of decline in lung function would be expected in individuals with lower nutritional status at baseline. Since plasma vitamin E is known to plateau with higher levels of supplementation and given that past trials of selenium indicated that effects of selenium were limited to participants with low baseline selenium(17), the consideration of individual nutriture at the study baseline is important for developing a fuller understanding of nutrient effects.

Finally, this dissertation addresses the effect of vitamin E supplementation on plasma vitamin E levels. Since any effect of vitamin E on health outcomes, including lung function and COPD, is proposed to act by transport of vitamin E through the plasma to the target tissue, individuals with a greater increase in plasma vitamin E in response to supplementation may experience a greater benefit from the intervention. It has been shown that plasma response to vitamin E supplementation is highly variable between individuals, but stable within an individual over time (thus, low intra-individual variability). For this reason, there is interest in identifying particular characteristics that may contribute to differential plasma response; such characteristics would also be

Figure 1. Epidemiologic Framework[1]



[1] This dissertation investigates the effects of selenium and vitamin E on the oxidative stress biomarker (urine F_2-Isoprostanes) and rate of decline in lung function. This diagram is simplified to show only vitamin E supplementation, since only vitamin E is addressed in relation to change in the nutrient biomarker (plasma alpha-tocopherol levels). Baseline nutrition is measured as baseline plasma nutrient levels (alpha-tocopherol and selenium).

expected to contribute to differential functional benefit from supplementation. Some cross-sectional associations have been reported, but the research reported herein addresses a gap in the literature by using a long-term randomized controlled trial with a high percentage of African Americans, who are far less well-studied, and indeed are notably absent from a prior large vitamin E trial(21). Furthermore, this research adds new information by a thorough consideration of both α- and γ-tocopherol.

While the research aims addressed in this dissertation focus on lung function phenotypes (rate of decline) and do not directly address the effect of nutritional and/or environmental factors on COPD, the oxidant-antioxidant balance is critical in maintaining lung health and an imbalance is proposed to be a key factor in COPD risk. Additionally, spirometry (pulmonary function testing) is the cornerstone of COPD diagnosis, and individuals with steeper rates of lung function decline are at greatest risk of developing COPD. Thus, any effects of these nutrients on oxidative stress and/or pulmonary function suggests the potential importance of these nutrients as preventives for COPD. Since COPD is an incurable disease with increasing prevalence and this dissertation addresses gaps in the literature, this dissertation contributes meaningful new information to the scientific body of knowledge about lung function, COPD, and oxidative stress.

References

1. Institute NHLaB. Morbidity and mortality: Chart book on cardiovascular, lung and blood diseases. 2009;November 8 2012. Internet: http://www.nhlbi.nih.gov/resources/docs/2009_ChartBook_508.pdf

2. Anthonisen NR, Connett JE, Murray RP. Smoking and Lung Function of Lung Health Study Participants after 11 Years. Am J Respir Crit Care Med 2002;166:675-9.

3. Croxton TL, Weinmann GG, Senior RM, Wise RA, Crapo JD, Buist AS. Clinical Research in Chronic Obstructive Pulmonary Disease: Needs and Opportunities. Am J Respir Crit Care Med 2003;167:1142-9.

4. Barnes PJ. Future Treatments. In: Currie, GP, ed. ABC of COPD. UK: John Wiley & Sons, 2011:72-75.

5. Butland BK, Fehily AM, Elwood PC. Diet, lung function, and lung function decline in a cohort of 2512 middle aged men. Thorax 2000;55:102-8.

6. Grievink L, Smit HA, Ocké MC, van 't Veer P, Kromhout D. Dietary intake of antioxidant (pro)-vitamins, respiratory symptoms and pulmonary function: the MORGEN study. Thorax 1998;53:166-71.

7. Hu G, Cassano PA. Antioxidant Nutrients and Pulmonary Function: The Third National Health and Nutrition Examination Survey (NHANES III). American Journal of Epidemiology 2000;151:975-81.

8. Hu G, Zhang X, Chen J, Peto R, Campbell TC, Cassano PA. Dietary Vitamin C Intake and Lung Function in Rural China. American Journal of Epidemiology 1998;148:594-9.

9. Ochs-Balcom HM, Grant BJB, Muti P, et al. Antioxidants, oxidative stress, and pulmonary function in individuals diagnosed with asthma or COPD. European Journal of Clinical Nutrition 2006;60:991--999.

10. Tabak C, Smit HA, Heederik D, Ocké MC, Kromhout D. Diet and chronic obstructive pulmonary disease: independent beneficial effects of fruits, whole grains, and alcohol (the MORGEN study). Clinical & Experimental Allergy 2001;31:747-55.

11. McKeever TM, Lewis SA, Cassano PA, et al. Patterns of dietary intake and relation to respiratory disease, forced expiratory volume in 1 s, and decline in 5-y forced expiratory volume. The American Journal of Clinical Nutrition 2010;92:408-15.

12. Cassano PA, Guertin KA, Kristal AR, et al. A Randomized Controlled Trial of Vitamin E and Selenium on Rate of Decline in Lung Function; in preparation.

13. Lippman SM, Goodman PJ, Klein EA, et al. Designing the Selenium and Vitamin E Cancer Prevention Trial (SELECT) J Natl Cancer Inst 2005;97:94-102.

14. Otten JJ, Hellwig JP, Meyers LD. Dietary reference intakes : the essential guide to nutrient requirements. Washington, DC: National Academies Press, 2006.

15. Kelleher J, Losowsky MS. The absorption of α-tocopherol in man. British Journal of Nutrition 1970;24:1033-47.

16. Brenneisen P, Steinbrenner H, Sies H. Selenium, oxidative stress, and health aspects. Mol Aspects Med 2005;26:256-67.

17. Rayman MP. Selenium and human health. The Lancet 379:1256-68.

18. Agler AH, Kurth T, Gaziano JM, Buring JE, Cassano PA. Randomised vitamin E supplementation and risk of chronic lung disease in the Women's Health Study. Thorax 2011;66:320-5.

19. Cassano PA, Arnold KB, Guertin KA, et al. Effect Of Vitamin E And Selenium On Incidence Of Physician-Diagnosed COPD: The Selenium And Vitamin E Cancer Prevention Trial (SELECT); in preparation.

20. Heart Protection Study Collaborative Group. MRC/BHF Heart Protection Study of antioxidant vitamin supplementation in 20 536 high-risk individuals: a randomised placebo-controlled trial. Lancet 2002;360:23.

21. Rautalahti M, Virtamo J, Haukka J, et al. The Effect of Alpha-Tocopherol and Beta-Carotene Supplementation on COPD Symptoms. Am J Respir Crit Care Med 1997;156:1447-52.

22. Mannino DM, Homa DM, Akinbami LJ, Ford ES, Redd SC. Chronic obstructive pulmonary disease surveillance - United States, 1971-2000. Respir Care 2002;47:1184-99.

23. Mannino DM, Gagnon RC, Petty TL, Lydick E. Obstructive Lung Disease and Low Lung Function in Adults in the United States: Data From the National Health and Nutrition Examination Survey, 1988-1994. Arch Intern Med 2000;160:1683-9.

24. Pelkonen M, Notkola IL, Tukiainen H. Smoking cessation, decline in pulmonary function and total mortality: a 30 year follow up study among the Finnish cohorts of the Seven Countries Study. Thorax 2001;56:703-7.

25. Schunemann HJ, Grant BJB, Freudenheim JL, et al. The Relation of Serum Levels of Antioxidant Vitamins C and E, Retinol and Carotenoids with Pulmonary Function in the General Population. Am J Respir Crit Care Med 2001;163:1246-55.

26. Kuller LH, Ockene JK, Townsend M, Browner W, Meilahn E, Wentworth DN. The epidemiology of pulmonary function and COPD mortality in the multiple risk factor intervention. trialThe American review of respiratory disease 1989;140:

27. Lange P, Nyboe J, Appleyard M, Jensen G, Schnohr P. Spirometric findings and mortality in never-smokers. J Clin Epidemiol 1990;43:867-73.

28. Tockman MS, Comstock GW. Respiratory risk factors and mortality: longitudinal studies in Washington County, Maryland Am Rev Respir Dis 1989;140:S56-63.

29. Beaty TH, Cohen BH, Newill CA, Menkers HA, Diamond EL, Chen CJ. Impaired pulmonary function as a risk factor for mortality. American Journal of Epidemiology 1982;116:102-13.

30. Kadiiska MB, Gladen BC, Baird DD, et al. Biomarkers of Oxidative Stress Study II: Are oxidation products of lipids, proteins, and DNA markers of CCl4 poisoning? Free Radical Biology and Medicine 2005;38:698-710.

CHAPTER 2

EFFECT OF VITAMIN E AND SELENIUM ON URINE F_2-ISOPROSTANES, A BIOMARKER OF OXIDATIVE STRESS

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Abstract

Introduction: Oxidative stress contributes to rate of decline in lung function, specifically forced expiratory volume in the first second (FEV_1), and progression of chronic obstructive pulmonary disease (COPD). Current cigarette smokers without COPD were studied to test the hypothesis that supplementation with nutrients with antioxidant properties mitigates oxidative stress, which leads to functional effects on lung health manifested as an attenuated rate of decline in FEV_1.

Methods: In a randomized placebo controlled trial of vitamin E (400 IU/d *all rac*-alpha-tocopheryl acetate) and selenium (200 μg/d L-selenomethionine), urine 8-iso-prostaglandin $F_{2\text{-alpha}}$ (F2-IsoPs), an oxidative stress biomarker, was measured in 312 current smokers after thirty-six months on study. The effect of supplements on F2-IsoPs and the mediation of supplement effects on lung function by F2-IsoPs were investigated.

Results: Compared to placebo, vitamin E, alone and combined with selenium, lowered urine F2-IsoPs by 21% (p=0.0231) and 9% (p=0.3738), respectively; there was no effect of selenium alone. Higher F2-IsoPs were associated with lower FEV_1, (β=-95 mL per unit increase in lnF2-IsoP; p=0.0679), although there was no evidence of an association with rate of decline in FEV_1 (p=0.7922). Treatment with vitamin E attenuated rate of decline in FEV_1 by 22 mL/year (p=0.1453), but there was no evidence that this effect was mediated by lowering of urine F2-IsoPs.

Conclusions: Vitamin E decreases systemic oxidative stress, however, a tissue-specific biomarker may be needed to fully understand the mechanisms underlying the effect of vitamin E supplementation on lung function in smokers.

Introduction

Oxidative stress, an imbalance between pro-oxidants and antioxidants, is hypothesized to play a role in the pathogenesis of chronic obstructive pulmonary disease (COPD). COPD is characterized by increased inflammation and irreversible airflow limitation caused by lung tissue damage. Cigarette smoking, the primary risk factor for COPD, generates reactive oxygen species (ROS), which oxidize membrane phospholipids and lead to tissue damage and/or cell death(1). Oxidants, including those from cigarette smoke, also play a role in the molecular mechanisms that control lung inflammation(2).

F_2-isoprostanes (F2-IsoPs) are the gold-standard biomarker of *in vivo* oxidative stress(3); these prostaglandin-like bioactive compounds are biosynthesized *in vivo* by free-radical-catalyzed lipid peroxidation of arachidonic acid(4). 8-iso-prostaglandin $F_{2\text{-alpha}}$ (8-iso-PGF2α, 15-F_{2t}-isoprostane) is a chemically stable isomer of F2-IsoPs and a reliable marker of oxidative stress in urine and plasma(5); elevations are associated with a wide range of diseases(6), and F2-IsoPs increase with smoking(7, 8) and COPD(9-12). Heritability of F2-IsoPs is estimated at 10%(13), thus environmental factors are the primary determinant of inter-individual variability in F2-IsoPs.

Observational studies consistently report that diets high in fruits and vegetables reduce the risk of chronic disease(14), and studies of lung outcomes are consistent with this overall trend. Thus, increased consumption of antioxidant-rich foods and higher concentrations of serum biomarkers of nutrients with antioxidant properties are associated with fewer COPD symptoms and better lung function(15, 16). Assuming antioxidant nutrients contribute to a causal mechanism lowering the risk of poor lung outcomes, establishing that nutrient intake leads to lower oxidative stress would provide

important data for causal inference. Prior studies do not comprehensively investigate the effect of antioxidant nutrients on a biomarker of lipid peroxidation such as F2-IsoPs in the context of a causal chain leading to changes in outcomes.

Small vitamin E intervention studies assessing effects on F2-IsoPs report mixed results. Vitamin E had no effect on F2-IsoPs in five moderate smokers (given 100 IU/d, form unspecified) or seven heavy smokers (given 800 IU/d, form unspecified) over five days, but vitamin E in combination with vitamin C lowered F2-IsoPs by 22%(17). In forty-six cigarette smokers, vitamin E (at doses of 300, 600, and 1200 mg/d *all rac* alpha *(*α)-tocopheryl acetate) had no effect on F2-IsoPs during a three-week intervention(18). In a six-month randomized, double-blind, placebo-controlled trial in eighty overweight subjects, natural vitamin E (800 IU/d for three months, followed by 1200 IU/d for 3 months, all-natural *RRR* d-α-tocopherol), which has twice the bioavailability of synthetic vitamin E(19), reduced F2-IsoPs by 11%(20), suggesting the importance of trial duration and/or form of vitamin E supplement. In a randomized trial of two months duration, nonsmokers on vitamin E (800 IU/day, all-natural *RRR* d-α-tocopherol) had a 4% reduction in plasma F2-IsoPs, with greater reduction in individuals with higher baseline levels of oxidative stress(21), indicating the importance of potential to benefit in considering nutrient effects.

Selenium also has antioxidant properties and is increasingly recognized as an important constituent in protective enzymes and amino acids, including selenocysteine and selenomethionine(22). Higher selenium status and selenium supplementation are associated with better health outcomes, however results of prior intervention studies are mixed and benefits may be limited to individuals with low baseline selenium status(23).

Few published studies investigate selenium intervention in relation to F2-IsoPs. One observational study among Swedish men reported significantly lower plasma F2-IsoPs among men in the highest quartile of serum selenium(8), and a small (forty intensive care patients) short term (three day) clinical intervention study found no effect of high dose selenium (474, 316, or 158 μg/day intravenous selenium selenite) on plasma F2-IsoPs(24). In contrast, a small randomized study in eighty-one Australian men with low baseline selenium (mean 122 μg/L) found no effect of selenium-fortified wheat biscuits (wheat bio-fortified with selenium or wheat process fortified with selenomethionine, compared to normal wheat) on plasma F2-IsoPs, although the intervention significantly increased plasma selenium levels(25). Further investigation of the effect of selenium supplements on F2-IsoPs is needed to provide context for understanding the effect of selenium on health endpoints.

In summary, past randomized trials of antioxidant nutrient effects on biological markers of oxidative stress report mixed results, but inferences are limited by cross-sectional and non-randomized designs, small sample size, short duration, and exposure measurement error when dietary intake is used to assess nutritional status. We addressed this gap in knowledge using data from a large randomized study of vitamin E and selenium supplementation to attenuate lung function decline. Specifically, we hypothesized that supplementation lowers oxidative stress in cigarette smokers, and that the higher oxidative stress burden in this group confers greater potential to benefit from intervention. The primary endpoint of the trial was rate of decline in lung function, thus we further hypothesized that supplementation leads to changes in F2-IsoPs that, in turn, mediate the protective effect of supplementation on lung function.

Methods

Study Population

The Selenium and Vitamin E Cancer Prevention Trial (SELECT), a prostate cancer primary prevention trial, randomized 35,433 male participants ages fifty and older at 428 study sites in North America to four arms: 400 IU/day α-tocopherol (*all rac*-α-tocopheryl acetate, hereafter referred to as vitamin E) plus selenium placebo; vitamin E placebo plus 200 μg/day selenomethionine (L-selenomethionine, hereafter referred to as selenium); both active supplements; or, vitamin E placebo plus selenium placebo(26). The Respiratory Ancillary Study (RAS) to SELECT investigated the effect of intervention on rate of decline in lung function in 2,920 SELECT men; cigarette smokers were preferentially studied in the RAS given their higher risk of steeper lung function decline(27). A urine sample at thirty-six months on study was obtained from a subset of participants, focusing primarily on cigarette smokers without COPD (n=312), although a few urine samples from never smokers without COPD (n=32) were collected and assayed for reference values. Urine samples from men with a self-report of physician-diagnosed COPD at study baseline (n=107) were also collected to explore the effect of intervention in men with prevalent lung disease.

Measurements

ELISA Measurement of Urine F_2-isoprostanes

F2-IsoPs were assayed in prepared urine samples using a competitive enzyme linked immunoassay (ELISA) kit from Cayman Chemicals (Ann Arbor, MI), following manufacturer instructions (supplemental methods). The between-run precision for a set of

control samples assayed over six months was estimated by the coefficient of variation, which was 18.7%.

Urine Creatinine

Creatinine was measured in urine samples using an ATAC 8000 (Vital Diagnostics, Lincoln, RI) and, later, a Siemens (Deerfield, IL) Dimension Xpand Plus according to the manufacturers' recommended methods. The published coefficients of variation for the Xpand are 2.5% and 4.2% for within- and between-run precision, respectively. Creatinine values from both devices were in excellent agreement, with an average difference of 2.3%.

Lung Function

Spirometry was conducted following the American Thoracic Society (ATS) guidelines(28). All pulmonary function measurements were carried out using a handheld electronic spirometer (EasyOne, ndd Medizintechnik, MA); pulmonary function testing was performed about once per year on each participant over at least three years. Lung parameters of interest included forced expiratory volume in the first second (FEV_1), and forced expiratory flow between 25 and 75 percent of the forced vital capacity (FEF_{25-75}). All pulmonary function test data were reviewed weekly, and identification of measurement problems triggered immediate remedial action. Only FEV_1 measurements with high reliability (repeated measurements $\leq$ 150 mL apart, the current ATS standard(28)) contributed to the models. Rate of decline estimates were calculated for participants whose repeated lung function measurements were $\geq$ 24 months apart (first to last test); only the first lung function measurement was used for participants not meeting this criterion.

Statistical Methods

All analyses were carried out in SAS, version 9.3 (SAS Institute, Cary, NC). Age, height, smoking dose, F2-IsoPs, and lung function were continuous variables, and smoking status, race/ethnicity, and treatment arm were categorical variables. The main analyses were intent-to-treat, preserving randomization to reduce confounding bias. One participant with an F2-IsoP value well out of range, and of questionable validity, was excluded from all analyses. Natural log (ln) transformation was used to normalize the distribution of F2-IsoPs.

Generalized linear regression models estimated the effect of intervention on F2-IsoPs; robust regression models were used to understand the influence of extreme, but valid, F2-IsoP values. Given the similarity of vitamin E effects in the vitamin E only and vitamin E plus selenium arms, further analyses considered the effect of any vitamin E, combining the two groups. Hierarchical linear regression models (mixed models) estimated the effect of intervention on repeated measurements of lung function, and in these models, mediation by F2-IsoPs was tested. In a sensitivity analysis, which was an as-treated analysis, the sample was limited to men adherent to study supplement at the visit closest to the urine collection date; adherence was defined as taking at least eighty percent of study supplement, according to pill counts.

Different variance-covariance matrices were investigated in the full RAS pulmonary function dataset (2,920 men), and variance components was selected as the best matrix based on degrees of freedom and comparison of AIC criteria. The Kenward-Rodgers method for standard error and denominator degrees-of-freedom (ddfm) correction was selected (ddfm=kr) for computing the denominator degrees of freedom for the tests of fixed effects. No discernable pattern was detected in plots of studentized residuals versus

predicted values, and there was no difference in residuals by treatment arm. In a sensitivity analysis including outlying observations, there was no difference in parameter estimates compared to those from the edited dataset, which excluded outliers. Refer to the Appendix for further details.

Results

At study baseline, participant characteristics in cigarette smokers with urine samples and lung function measurements were evenly distributed by treatment arm (Table 1). Approximately 50% of men were African American, and the mean age overall was 64 years. F2-IsoP values were highly variable in smokers (27-2218 pg/mg creatinine) and in men with COPD (52-1875 pg/mg creatinine, Supplemental Table 1). Unadjusted F2-IsoPs at thirty-six months on supplement differed by arm; in both smokers and men with COPD, men in the vitamin E arms had lower mean F2-IsoP values. Average nutrient levels of vitamin E (α-tocopherol) and selenium at baseline were 22.1 μmol/L (SD 9.8) and 170.7 ng/mL (SD 27.7), respectively (data not shown; no difference by arm).

Given that F2-IsoPs were assayed in samples collected after thirty-six months on supplement (thus no baseline F2-IsoP data are available), and assuming an intervention effect on the biomarker, the relation of participant characteristics to urine F2-IsoPs was considered only for the placebo arm (Table 2). Current smokers and men with COPD (Table 2 and Supplemental Table 3, respectively) had similar urine F2-IsoP concentrations, and, in comparison, values were 50% lower among disease-free never smokers (mean 222 pg/mg creatinine; Supplemental Table 2). Current smokers with COPD had the highest concentration of urine F2-IsoPs, with a mean concentration twice as high as disease-free

current smokers (809 vs. 461 pg/mg creatinine, Supplemental Table 3 and Table 2, respectively). Among current smokers in the placebo arm, African Americans had 30% higher urine F2-IsoPs compared to other race/ethnicities (mostly Caucasian). Increased smoking dose was associated with elevated F2-IsoPs, and concentrations also increased with age; the trend with age was best reflected by the median given the influence of extreme observations on the mean in the younger age categories. Contrary to expectation, higher body mass index (BMI), an indicator of adiposity, was associated with lower average and median F2-IsoPs.

Vitamin E and Urine F2-IsoPs

In cigarette smokers, treatment with vitamin E alone decreased urine F2-IsoPs by 21% (p=0.0231); the effect was attenuated in the combination arm (vitamin E plus selenium), with a 9% reduction, and was not statistically significant (Table 3). There was no statistically significant effect of selenium on F2-IsoPs; the direction of the effect was unexpectedly positive, indicating an increase in oxidative stress with supplementation. In a sensitivity analysis, limiting the sample to men adherent to study supplement (data not shown), the magnitude of the vitamin E effect increased; vitamin E was associated with a 27% reduction in F2-IsoPs (p=0.0115). The effect of selenium, alone and in combination with vitamin E, was unchanged in the sensitivity analysis, and neither effect was statistically significant.

When both vitamin E arms were combined into a single 'any' vitamin E group (alone or in combination with selenium), the intervention reduced the biomarker by 16% compared to the placebo group (β = -0.160, p=0.0669). In the sensitivity analysis including only adherent men, the magnitude of effect increased, and any vitamin E reduced urine F2-IsoPs

by 19% compared to placebo (β=-0.186, p=0.872, data not shown). Adjusting for smoking dose had little or no effect on the regression coefficients for treatment, but smoking dose was a significant independent predictor of urine F2-IsoPs (β=0.004, p=0.0351, in model testing effect of treatment). Furthermore, there was no evidence of an interaction between smoking dose and treatment (Type III P-value=0.6822) in relation to effects on F2-IsoPs.

F2-IsoPs and FEV_1 Rate of Decline

The effect of supplementation on a functional outcome, lung function decline, which is reported elsewhere for the RAS sample(27), was also investigated in this smoking subsample of the RAS (Table 4). Models with and without adjusting for smoking dose were considered to address over-control, given smoking leads to higher F2-IsoPs, but effects were similar thus models unadjusted for smoking dose are described below.

In current smokers the annual rate of decline in FEV_1 was 38 mL/year. Vitamin E alone attenuated the rate of decline (p=0.1555). The combination of vitamin E plus selenium also attenuated rate of decline in FEV_1, but to a lesser extent (Type III p-value for treatment categorical variable = 0.4470). While higher F2-IsoPs were associated with lower cross-sectional FEV_1 (p=0.0679), there was no association of F2-IsoPs with FEV_1 rate of decline (p=0.7922). In the sensitivity analysis limited to participants adherent to study supplement, vitamin E effects on lung function decline were similar (reduced decline by 20 mL/yr, p=0.2897; data not shown).

While vitamin E reduced F2-IsoPs and attenuated rate of decline in FEV_1, there was no evidence that the effect of vitamin E on rate of decline was mediated by F2-IsoPs; thus, in models estimating the effect of treatment on lung function decline, the coefficient for the

time by treatment interaction term (the effect of treatment on rate of decline) did not change appreciably when F2-IsoPs were added to the model.

In the small subgroup of men with COPD (n=107), a similar set of analyses was conducted (Supplemental Tables 1, 3-8). While the small sample size precludes definitive conclusions, results were consistent with an effect of vitamin E to lower oxidative stress, as reflected by urine F2-IsoPs. In contrast to the findings in current smokers, a similar effect of half the magnitude was observed for selenium supplementation, and the greatest reduction in oxidative stress was in the combined arm (F2-IsoPs lowered by 21%; p=0.2381).

A second outcome, FEF_{25-75}, which is a marker of small airways function, was also considered in analyses of current smokers (Supplemental Table 7), and the findings were similar to the FEV_1 endpoint in that there was no evidence that F2-IsoPs mediated an effect of treatment on FEF_{25-75}.

Discussion

This large, randomized intervention study permits a strong test of the hypothesis that antioxidant nutrients lower systemic oxidative stress as reflected by the F2-IsoP biomarker. Previous studies reporting mixed effects of vitamin E on F2-IsoPs were limited by sample size, study duration, self-reported dietary intake, and non-randomized designs. The randomized controlled trial reported herein provides definitive evidence that supplemental long-term synthetic α-tocopherol lowers oxidative stress, as reflected by the urine F2-IsoP biological marker. While higher F2-IsoPs were associated with a lower FEV_1, there was no evidence of an association with rate of decline in FEV_1. In this study of current

smokers, while treatment with vitamin E attenuated rate of decline in FEV_1, there was no evidence that this effect was mediated by lowering systemic F2-IsoPs.

No prior randomized controlled trials investigate the effects of long-term antioxidant supplementation, including vitamin E and selenium, on F2-IsoPs. Furthermore, no prior studies investigate the relation between lowering an oxidative stress biomarker and change in a functional outcome such as lung function. A recent observational study reported that urine F2-IsoPs were independently associated with mortality in patients with pulmonary arterial hypertension, underscoring the potential importance and utility of this biomarker in predicting lung health outcomes(29). Previous randomized controlled trials of antioxidant supplements and respiratory disease report mixed results(30-33), with most reporting no effect of vitamin E on lung disease outcomes. The current study is unique given the focus on prevention of lung function decline rather than disease, and the objective measurements collected, namely lung function and F2-IsoPs, are not subject to reporting bias as are the self-reported disease outcomes used in prior studies.

While there was no evidence that F2-IsoPs mediated the effect of vitamin E on rate of decline in FEV_1, the lack of mediation may be due to design issues. Although urine F2-IsoPs are a well-accepted biomarker of systemic oxidative stress that reflects whole-body processes, a lung-specific biomarker may be needed to identify mediation of effects on lung function. Furthermore, a single measure of F2-IsoPs at thirty-six months on study may not be indicative of usual, long-term values; change in F2-IsoPs from baseline to 36 months on study might have been more informative. Finally, another possibility is that vitamin E effects on lung function are mediated through pathways other than oxidative stress.

The vitamin E plus selenium effects are lower than the effect of vitamin E alone, for both the oxidative stress and the lung function endpoints. While there is no known mechanism through which selenium would reduce the antioxidant effects of vitamin E, and although selenium is a key component in antioxidant enzymes, effects in a selenium-replete sample such as the RAS(34) are less well understood. Selenium may have pro-oxidant activity at higher doses, but selenomethionine, the form of selenium studied herein, is not thought to have such pro-oxidant activity(35, 36). In this sample of cigarette smokers the effect of selenium alone was not statistically significant, but coefficients were consistent with an effect of selenium to increase urine F2-IsoPs in comparison to placebo, and the F2-IsoP-lowering effects were attenuated in the combination arm compared to the vitamin E alone arm. Thus, a pro-oxidant effect of selenium in this study cannot be ruled out.

The choice of urine F2-IsoPs was deliberate, as this biomarker is more stable than other biomarkers of oxidative stress and it reflects free radical lipid peroxidation(3). There is minimal diurnal variation in F2-IsoP levels(37), and therefore spot urine collections, as used herein, sufficiently capture a representative daily F2-IsoP value. While other studies measure plasma F2-IsoPs, urine F2-IsoPs are proposed to more accurately reflect *in vivo* lipid peroxidation since the higher lipid content in plasma results in *ex vivo* production of F2-IsoPs. Thus, plasma samples require immediate and rigorous processing for accurate assessment of F2-IsoP levels, whereas urine samples can be stored for long periods of time, and measurement is less affected by variation in sample handling(14). Additionally, plasma F2-IsoPs, due to rapid clearance, only reflect a brief period of time(38), but urine F2-IsoPs are a more time-integrated marker. There is some disagreement on whether ELISA is less accurate than gas chromatography/mass spectroscopy (GC/MS) for F2-IsoP

measurement(39), however our key comparisons require the correct rank ordering of samples, not detection of the most accurate actual value, minimizing any concerns about the use of ELISA.

A few small vitamin E intervention studies reported lowering of oxidative stress biomarkers, other than urine F2-IsoPs, in individuals with COPD(40, 41). As reviewed by Tsigliani et al, prior studies of antioxidant supplementation, including vitamin E, in COPD patients did not detect an effect of supplements on lung function(42). However, most prior studies were of short duration, small sample size, measured lower quality biomarkers, and did not assess rate of decline. Only one longitudinal study of COPD patients investigated the relation of systemic biomarkers to rate of decline in FEV_1, but F2-IsoPs were not investigated and none of the measured biomarkers predicted rate of decline(43). Due to limited numbers (n=105), our analyses of participants with COPD were exploratory. Smokers with COPD had less steep decline compared to never smokers with COPD, although at baseline men with COPD had much lower FEV_1 compared to COPD-free individuals. Vestbo et al report a "burn out" in rate of decline in COPD patients(43), which may explain the trends seen in this study. In participants with COPD, although no effect of supplement on rate of decline in lung function was observed, all supplements led to lowering of urine F2-IsoPs, and, in contrast to findings in disease-free smokers, the greatest reduction was in the combination arm.

While both nutrients studied have clear antioxidant functions, selenium and vitamin E may, under certain circumstances, act as pro-oxidants(35, 36, 44). When α-tocopherol reacts with reactive oxygen species in its antioxidant capacity, α-tocopherol radicals are formed, which can induce lipid peroxidation if the radicals are not reduced by co-

antioxidants such as vitamin C(44). Thus, associations between vitamin E and health endpoints may differ between observational studies, where diets high in vitamin E are likely to be similarly high in co-antioxidant nutrients, and intervention studies, particularly if the intervention does not include a co-antioxidant capable of reducing the vitamin E radical. The dual nature of the intervention nutrients, which requires careful consideration of context, including participant nutriture prior to supplementation and/or the dose and form of supplement, deserves further study.

This study provides strong evidence that vitamin E lowers a urine biomarker of systemic oxidative stress in cigarette smokers. Given reports of potentially harmful effects of vitamin E supplementation(45, 46), the possible benefits of supplementation must be carefully considered in the context of balancing risks and benefits. To our knowledge, this is the largest epidemiologic study of the effects of vitamin E on an oxidative stress biomarker. The consistency of effects on F2-IsoPs in both vitamin E arms (E alone and E plus Se), and the increased effect magnitude in sensitivity analyses limited to adherent participants support a true effect of vitamin E to lower systemic oxidative stress in cigarette smokers.

Table 1. Characteristics of RAS Participants, by Study Arm, For Current Smoker Men Providing Urine Sample at 36 Months of Supplementation[a]

Variable:	Placebo	Vitamin E	Selenium	Vitamin E plus Selenium
Number	81	82	66	83
Age, years	62.9 ± 5.8	62.9 ± 6.3	63.6 ± 7.0	63.0 ± 5.7
Time on Study, months[b]	38.1 ± 6.7	37.9 ± 5.4	37.4 ± 5.1	38.8 ± 7.6
Body Mass Index (BMI), kg/m^2[c]	26.8 ± 4.4	27.5 ± 4.6	27.2 ± 3.7	27.2 ± 4.4
Height, cm[c]	177.6 ± 7.6	176.5 ± 7.6	175.6 ± 7.9	175.4 ± 7.3
Weight, kg[c]	84.9 ± 16.3	85.5 ± 14.1	84.6 ± 13.8	83.6 ± 14.7
Race				
African American	43 (53)	38 (46)	42 (64)	40 (48)
Caucasian/ Other	38 (47)	44 (54)	24 (36)	43 (52)
Cigarettes Smoked Per Day[d]				
<5/day	15 (19)	18 (22)	15 (23)	19 (23)
≥5/day	66 (81)	64 (78)	51 (77)	64 (77)
Urine 8-iso-PGF-$_{2\alpha}$ (pg/mg creatinine)	461.3 ± 355.7	354.0 ± 251.2	480.1 ± 305.4	408.6 ± 319.7
	347.0 (82.0-1767.6)[e]	281.3 (55.0-1366.3)	399.7 (27.5-1723.3)	343.8 (58.4-2218.1)

[a] RAS, Respiratory Ancillary to SELECT; SELECT, The Selenium and Vitamin E Cancer Prevention Trial. Current smokers include only men without Chronic Obstructive Pulmonary Disease (COPD). Urine samples collected from 9/2004 to 10/2008. Values in table are mean ± standard deviation or number (%) of participants. Values for weight, race, height, BMI are study baseline; all other variables describe participant status up to of date of urine collection

[b] Time on study at urine collection. By protocol, urine collected at 36 months on study; dates of urine collection ranged from 24-76 months on study.

[c] At study baseline

[d] Smoking dose for current smokers only

[e] Median (Range)

Table 2. Urine 8-iso-PGF-$_{2\alpha}$ (pg/mg creatinine) According to Demographic Characteristics Among Current Smokers in the Double Placebo Arm (n=81) of the RAS[a]

Characteristic	Mean (SD)	Median (Range)
Full Sample	461.3 (355.7)	347.0 (82.0-1767.6)
Age		
50-59	460.2 (345.7)	329.4 (90.8-1444.9)
60-69	477.6 (391.7)	348.4 (82.0-1767.6)
≥70	387.0 (183.6)	347.0 (129.7-677.8)
Ethnicity/Race		
African-American	516.5 (413.8)	389.5 (129.7-1767.6)
Caucasian/Other	398.9 (267.8)	331.9 (82.0-1291.4)
Smoking Dose		
<5 cigarettes/day	435.3 (379.3)	282.3 (90.8-1376.5)
≥5 cigarettes/day	467.2 (353.0)	363.8 (82.0-1767.6)
Body Mass Index (BMI), kg/m^2		
≤25	520.7 (404.4)	366.2 (98.8-1767.6)
≥25	418.4 (313.4)	334.1 (82.0-1712.0)

[a] RAS, Respiratory Ancillary to SELECT; SELECT, The Selenium and Vitamin E Cancer Prevention Trial. Current smokers include only men without Chronic Obstructive Pulmonary Disease (COPD).

Table 3. Linear Regression Estimates of the Effect of Treatment on Urine 8-iso-PGF-$_{2\alpha}$ in Current Cigarette Smokers in the RAS to SELECT[a]

	Placebo	Vitamin E	Selenium	Vitamin E plus Selenium
Model-derived Estimates:				
Predicted[b] urine 8-iso-PGF-$_{2\alpha}$, pg/mg creatinine	380	302	409	347
% change in urine 8-iso-PGF-$_{2\alpha}$[c]	Reference	-21%	8%	-9%
Model Results:				
Regression Coefficient[d]	Reference	-0.230	0.069	-0.090
SE[e]	-	0.101	0.107	0.101
P-value[f]	-	0.0231	0.5217	0.3738

[a] RAS, Respiratory Ancillary to SELECT; SELECT, The Selenium and Vitamin E Cancer Prevention Trial. Current smokers (n=312) include only men without Chronic Obstructive Pulmonary Disease (COPD). Least squares regression included treatment variables and adjusted for age and race; Type III P-value for set of treatment variables, p=0.0303
[b] ln(geometric mean)
[c] Percent (%) change in urine 8-iso-PGF-$_{2\alpha}$ concentration; values at 36 months for each treatment group compared to placebo/placebo reference group
[d]Regression coefficient conveys estimated difference in ln urine 8-iso-PGF-$_{2\alpha}$ concentration for each treatment group compared to placebo
[e] SE, standard error of regression coefficient
[f] P-value, Wald's test of regression coefficient

Table 4. Association of Lung Function (FEV_1) with Urine 8-iso-$PGF_{2\alpha}$ Estimated in Hierarchical Linear Regression Models for Current Cigarette Smokers in the RAS to SELECT[a]

		Vitamin E	Selenium	Vitamin E plus Selenium
Model-derived Estimates of Treatment[b] x Time Interaction[c]:				
Unadjusted	Regression Coefficient[d]	22.4	0.2	14.4
	SE[e]	15.4	16.5	15.4
	P-value[f]	0.1453	0.9892	0.3510
Adjusted[g,h]	Regression Coefficient[d]	22.0	1.0	14.0
	SE[e]	15.5	16.7	15.4
	P-value[f]	0.1555	0.9525	0.3641

[a] FEV_1, forced expiratory volume in the first second; RAS, Respiratory Ancillary to SELECT; SELECT, The Selenium and Vitamin E Cancer Prevention Trial. Current smokers, N=310, excluding cases of chronic obstructive pulmonary disease (COPD); regression models included treatment variables and adjusted for age and race; Type III P-value for set of treatment variables, unadjusted p=0.3937, adjusted p=0.4470

[b] Each treatment arm is compared to placebo/placebo, the reference group;

[c] Time (years), is the time variable in the mixed model; the coefficient for time conveys the average annual change in the lung function; the interaction of time * treatment conveys the effect of treatment on annual decline in lung function

[d] Regression coefficient conveys estimated difference in rate of decline in lung function for each treatment group compared to placebo. Model included age, height, race, time (years), treatment, F2-IsoPs, and 2-way interaction terms between time and smoking status, treatment, and F2-IsoPs. Urine F2-IsoPs were ln-transformed.

[e] SE, standard error of regression coefficient

[f] P-value, Wald's test of regression coefficient

[g] Adjusted model accounts for F2-IsoPs and interaction between time and F2-IsoPs

[h] lnF2-IsoPs β=-95.0 (51.9), p=0.0679; lnF2-IsoPs x time β=-2.3 (8.7), p=0.7922; mean rate of decline -37.5 mL/yr

References

1. Barnes PJ. Reactive oxygen species and airway inflammation. Free Radical Biology & Medicine 1990;9:235-243.

2. Macnee W. Oxidants/Antioxidants and COPD. Chest 2000;117:303S-17S.

3. Kadiiska MB, Gladen BC, Baird DD, et al. Biomarkers of Oxidative Stress Study II: Are oxidation products of lipids, proteins, and DNA markers of CCl4 poisoning? Free Radical Biology and Medicine 2005;38:698-710.

4. Morrow JD, Hill KE, Burk RF, Nammour TM, Badr KF, Roberts LJ. A series of prostaglandin F2-like compounds are produced in vivo in humans by a non-cyclooxygenase, free radical-catalyzed mechanism. Proceedings of the National Academy of Sciences 1990;87:9383-7.

5. Kadiiska MB, Gladen BC, Baird DD, et al. Biomarkers of oxidative stress study: are plasma antioxidants markers of CCl4 poisoning? Free Radical Biology and Medicine 2000;28:838-45.

6. Basu S. F_2-isoprostanes in human health and diseases: from molecular mechanisms to clinical implications. Antioxidants & Redox Signaling 2008;10:1405-34.

7. Morrow JD, Frei B, Longmire AW, et al. Increase in Circulating Products of Lipid Peroxidation (F2-Isoprostanes) in Smokers — Smoking as a Cause of Oxidative Damage. N Engl J Med 1995;332:1198-203.

8. Helmersson J, Ärnlöv J, Vessby B, Larsson A, Alfthan G, Basu S. Serum selenium predicts levels of F2-isoprostanes and prostaglandin F2α in a 27 year follow-up study of Swedish men. Free Radical Research 2005;39:763-70.

9. Pratico D, Basili S, Vieri M, Cordova C, Violi F, Fitzgerald GA. Chronic Obstructive Pulmonary Disease Is Associated with an Increase in Urinary Levels of Isoprostane F2alpha -III, an Index of Oxidant Stress. Am J Respir Crit Care Med 1998;158:1709-14.

10. Montuschi P, Collins JV, Ciabattoni G, et al. Exhaled 8-Isoprostane as an In Vivo Biomarker of Lung Oxidative Stress in Patients with COPD and Healthy Smokers. Am J Respir Crit Care Med 2000;162:1175-7.

11. Basu S, Helmersson J, Jarosinska D, Sällsten G, Mazzolai B, Barregård L. Regulatory factors of basal F2-isoprostane formation: Population, age, gender and smoking habits in humans. Free Radic Res 2009;43:85-91.

12. Biernacki WA, Kharitonov SA, Barnes PJ. Increased leukotriene B4 and 8-isoprostane in exhaled breath condensate of patients with exacerbations of COPD. Thorax 2003;58:294-8.

13. Schnabel RB, Lunetta KL, Larson MG, et al. The Relation of Genetic and Environmental Factors to Systemic Inflammatory Biomarker Concentrations. Circulation: Cardiovascular Genetics 2009;2:229-37.

14. Mayne ST. Antioxidant Nutrients and Chronic Disease: Use of Biomarkers of Exposure and Oxidative Stress Status in Epidemiologic Research. The Journal of Nutrition 2003;133:933S-40S.

15. Hu G, Cassano PA. Antioxidant Nutrients and Pulmonary Function: The Third National Health and Nutrition Examination Survey (NHANES III). American Journal of Epidemiology 2000;151:975-81.

16. Grievink L, Smit HA, Ocké MC, van 't Veer P, Kromhout D. Dietary intake of antioxidant (pro)-vitamins, respiratory symptoms and pulmonary function: the MORGEN study. Thorax 1998;53:166-71.

17. Reilly M, Delanty N, Lawson JA, FitzGerald GA. Modulation of Oxidant Stress In Vivo in Chronic Cigarette Smokers. Circulation 1996;94:19-25.

18. Patrignani P, Panara MR, Tacconelli S, et al. Effects of Vitamin E Supplementation on F2-Isoprostane and Thromboxane Biosynthesis in Healthy Cigarette Smokers. Circulation 2000;102:539-45.

19. Burton GW, Traber MG, Acuff RV, et al. Human plasma and tissue alpha-tocopherol concentrations in response to supplementation with deuterated natural and synthetic vitamin E. The American Journal of Clinical Nutrition 1998;67:669-84.

20. Sutherland WHF, Manning PJ, Walker RJ, de Jong SA, Ryalls AR, Berry EA. Vitamin E supplementation and plasma 8-isoprostane and adiponectin in overweight subjects. Obesity 2007;15:386-91.

21. Block G, Jensen CD, Morrow JD, et al. The effect of vitamins C and E on biomarkers of oxidative stress depends on baseline level. Free Radical Biology and Medicine 2008;45:377-84.

22. Brenneisen P, Steinbrenner H, Sies H. Selenium, oxidative stress, and health aspects. Mol Aspects Med 2005;26:256-67.

23. Rayman MP. Selenium and human health. The Lancet 379:1256-68.

24. Mishra V, Baines M, Perry SE, et al. Effect of selenium supplementation on biochemical markers and outcome in critically ill patients. Clinical Nutrition 2007;26:41-50.

25. Wu J, Salisbury C, Graham R, Lyons G, Fenech M. Increased consumption of wheat biofortified with selenium does not modify biomarkers of cancer risk, oxidative stress, or immune function in healthy Australian males. Environ Mol Mutagen 2009;50:489-501.

26. Lippman SM, Goodman PJ, Klein EA, et al. Designing the Selenium and Vitamin E Cancer Prevention Trial (SELECT) J Natl Cancer Inst 2005;97:94-102.

27. Cassano PA, Guertin KA, Kristal AR, et al. A Randomized Controlled Trial of Vitamin E and Selenium on Rate of Decline in Lung Function; in preparation.

28. American Thoracic Society. Standardisation of spirometry. European Respiratory Journal 2005;26:319-38.

29. Cracowski J, Degano B, Chabot F, et al. Independent Association of Urinary F2-Isoprostanes With Survival in Pulmonary Arterial Hypertension. Chest 2012;142:869-76.

30. Agler AH, Kurth T, Gaziano JM, Buring JE, Cassano PA. Randomised vitamin E supplementation and risk of chronic lung disease in the Women's Health Study. Thorax 2011;66:320-5.

31. Cassano PA, Arnold KB, Guertin KA, et al. Effect Of Vitamin E And Selenium On Incidence Of Physician-Diagnosed COPD: The Selenium And Vitamin E Cancer Prevention Trial (SELECT); in preparation.

32. Heart Protection Study Collaborative Group. MRC/BHF Heart Protection Study of antioxidant vitamin supplementation in 20 536 high-risk individuals: a randomised placebo-controlled trial. Lancet 2002;360:23.

33. Rautalahti M, Virtamo J, Haukka J, et al. The Effect of Alpha-Tocopherol and Beta-Carotene Supplementation on COPD Symptoms. Am J Respir Crit Care Med 1997;156:1447-52.

34. Lippman SM, Klein EA, Lippman SM, et al. Effect of Selenium and Vitamin E on Risk of Prostate Cancer and Other Cancers: The Selenium and Vitamin E Cancer Prevention Trial (SELECT). JAMA 2009;301:39-51.

35. Spallholz JE. On the nature of selenium toxicity and carcinostatic activity. Free Radical Biology and Medicine 1994;17:45-64.

36. Stewart MS, Spallholz JE, Neldner KH, Pence BC. Selenium compounds have disparate abilities to impose oxidative stress and induce apoptosis. Free Radical Biology and Medicine 1999;26:42-8.

37. Helmersson H, Basu S. F2-Isoprostane excretion rate and diurnal variation in human urine. Prostaglandins, Leukotrienes and Essential Fatty Acids 1999;61:203-5.

38. Nourooz-Zadeh J. Key issues in F2-isoprostane analysis. Biochem Soc Trans 2008;36:1060-5.

39. Il'yasova D, Morrow JD, Ivanova A, Wagenknecht LE. Epidemiological marker for oxidant status: comparison of the ELISA and the gas chromatography/mass spectrometry assay for urine 2,3-dinor-5,6-dihydro-15-F2t-isoprostane. Ann Epidemiol 2004;14:793-7.

40. Agacdiken A, Basyigit I, Özden M, et al. The effects of antioxidants on exercise-induced lipid peroxidation in patients with COPD. Respirology 2004;9:38-42.

41. Daga MK, Chhabra R, Sharma B, Mishra TK. Effects of Exogenous Vitamin E Supplementation on the Levels of Oxidants and Antioxidants in Chronic Obstructive Pulmonary Disease. Journal of Bioscience 2003;28:7-11.

42. Tsiligianni IG, van der Molen T. A systematic review of the role of vitamin insufficiencies and supplementation in COPD. Respiratory Research 2010;11:171.

43. Vestbo J, Edwards LD, Scanlon PD, et al. Changes in FEV1 over time in COPD. New England Journal of Medicine 2011;365:1184-92.

44. Rietjens IM, Boersma MG, Haan L, et al. The pro-oxidant chemistry of the natural antioxidants vitamin C, vitamin E, carotenoids and flavonoids. Environ Toxicol Pharmacol 2002;11:321-33.

45. Klein E, Thompson I, Tangen C, et al. Vitamin E and the risk of prostate cancer: The selenium and vitamin E cancer prevention trial (SELECT). The Journal of the American Medical Association 2011;306:1549-56.

46. The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group. The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. N Engl J Med 1994;330:1029-35.

ONLINE SUPPLEMENTAL MATERIALS

Supplemental Methods:

Measurements

Purification of urine samples

Urine specimens were collected at in-person SELECT visits after thirty-six months on study supplement using a "clean catch" method into sterile urine collection containers. Specimens were sealed and shipped overnight in an insulated container with ice to Cornell University. A single technician performed all F2-IsoP and creatinine assays. Upon arrival at Cornell, urine samples were pipetted into cryovials in 2 mL aliquots then immediately frozen at -80^{o}C. Once all specimens were collected, samples were thawed at room temperature, centrifuged to remove particulates, then 1 mL aliquots were added to microcentrifuge tubes. Addition of 200 μL of sorbent to the microcentrifuge tubes was followed by gentle mixing for 45 minutes. The tubes were then centrifuged to sediment out the sorbent and the supernatant was removed and disposed of. The remaining sorbent was washed with 1 mL Nanopure water then centrifuged with removal and disposal of the supernatant. The washing procedure was repeated. The washed sorbent was then resuspended in 0.5 mL Eicosanoid Affinity Column Elution Buffer (Fisher Scientific) and briefly vortexed. The sample was then centrifuged and the elution buffer was removed and saved. The resuspension was repeated and the elution buffer washes were all combined and dried down overnight on a speed vacuum. Dried samples were then dissolved in 1 mL of EIA buffer (Cayman Chemicals).

ELISA measurement of F_2-isoprostanes

F2-IsoPs were assayed according to manufacture directions. Dilute EIA and wash buffers were prepared with Nanopure water. Eight standard samples were prepared by mixing with EIA buffer with the standard using serial dilutions. The 500 dtn tracer was reconstituted with 30 mL of EIA buffer and the 500 dtn antiserum was reconstituted with 30 mL of EIA buffer. Plate set up was performed per the Cayman Chemical suggested method. After all standards, samples, tracers and antiserum aliquots were added to the plate, the plate was covered and incubated for 18 hours at room temperature. The plate was developed by reconstituteing 250 dtn of Ellman's Reagent with 50 mL of Nanopure water. The plate was washed 5 times with wash buffer solution. To each well of the plate, 200 µL of Ellman's Reagent was added and 5 µL of tracer was added to total activity wells. The plate was then covered and the plates were developed on a shaker in the dark. The plate was read at 405 nm wavelength after 60 minutes and 90 minutes of development.

Supplemental Table 1 Characteristics of RAS Participants, by Study Arm, For Men with COPD Providing Urine Sample at 36 Months of Supplementation[a]

Variable:	Placebo	Vitamin E	Selenium	Vitamin E + Selenium
Number	23	25	31	28
Age, years	68.9 ± 7.7	66.0 ± 7.8	64.6 ± 8.0	66.4± 7.4
Time on Study, months[b]	39.7 ± 7.7	37.2 ± 2.8	38.1 ± 7.7	36.5 ± 0.5
Body Mass Index (BMI), kg/m^2 [c]	26.4 ± 4.8	28.9 ± 6.5	26.4 ± 4.7	27.6 ± 5.2
Height, cm[c]	176.1 ± 6.9	173.7 ± 5.1	178.7± 8.2	177.7 ± 7.0
Weight, kg[c]	82.1 ± 15.4	88.1 ± 19.4	85.6 ± 17.2	87.4 ± 12.7
Race				
African American	9 (39)	9 (36)	18 (58)	12 (43)
Caucasian/ Other	14 (61)	16 (64)	13 (42)	16 (57)
Cigarette Smoking				
Never	4 (17)	4 (16)	3 (10)	1 (4)
Former	11 (48)	12 (48)	10 (32)	11 (40)
Current	8 (35)	9 (36)	18 (58)	16 (57)
Cigarettes Smoked Per Day[d]				
<5/day	1 (4)	1 (4)	2 (6)	0 (0)
≥5/day	7 (30)	8 (32)	16 (51)	16 (57)
Urine 8-iso-PGF-$_{2\alpha}$ (pg/mg creatinine)	449.5 ± 407.9	391.9 ± 445.7	442.6 ± 317.8	386.2 ± 317.8
	330.2 (81.9-1677.9)[e]	246.6 (102.6-1874.5)[e]	358.0 (108.3-1236.9)[e]	293.6 (52.2-1434.4)[e]

[a] RAS, Respiratory Ancillary to SELECT; SELECT, The Selenium and Vitamin E Cancer Prevention Trial; COPD, Chronic Obstructive Pulmonary Disease; samples collected from 9/2004 to 10/2008. Values in table are mean ± standard deviation or number (%) of participants. Values for weight, race, height, BMI are study baseline; all other variables describe participant status up to of date of urine collection

[b] Time on study at urine collection. By protocol, urine collected at 36 months on study; dates of urine collection ranged from 35-79 months on study.

[c] At study baseline

[d] Smoking dose for current smokers only

[e] Median (Range)

Supplemental Table 2 Characteristics of RAS Participants, by Study Arm, for Never Smoker Men without COPD Providing Urine Sample at 36 Months of Supplementation[a]

Variable:	Placebo	Vitamin E	Selenium	Vitamin E + Selenium
Number	7	12	7	6
Age, years	66.1(9.3)	64.4 (7.5)	67.8 (9.9)	61.9 (4.8)
Time on Study, months[b]	34.6 (5.3)	44.0 (12.5)	45.1 (15.7)	38.1 (2.4)
Body Mass Index (BMI), kg/m^2 [c]	32.1 (2.1)	29.4 (4.7)	26.6 (2.0)	26.0 (3.1)
Height, cm[c]	178.0 (7.2)	172.0 (7.5)	174.4 (6.4)	175.8 (8.0)
Weight, kg[c]	101.1 (10.5)	87.9 (13.8)	83 (5.8)	78.7 (9.4)
Race				
African American	3 (43)	4 (33)	2 (29)	3 (50)
Caucasian/Other	4 (57)	8 (67)	5 (71)	5 (83)
Urine 8-iso-PGF-$_{2\alpha}$ (pg/mg creatinine)	221.6 (132.4)	197.1 (151.6)	217.6 (177.1)	164.3 (87.2)
	181.0 (103.7, 469.3)[d]	131.8 (64.4, 530.8)[d]	147.0 (69.5, 591.4)[d]	159.0 (67.9, 322.6)[d]

[a] RAS, Respiratory Ancillary to SELECT; SELECT, The Selenium and Vitamin E Cancer Prevention Trial; COPD, Chronic Obstructive Pulmonary Disease; samples collected from 9/2004 to 10/2008. Values in table are mean ± standard deviation or number (%) of participants. Values for weight, race, height, BMI are study baseline; all other variables describe participant status up to of date of urine collection

[b] Time on study at urine collection. By protocol, urine collected at 36 months on study; dates of urine collection ranged from 23-79 months on study.

[c] At study baseline

[d] Median (Range)

Supplemental Table 3 Urine 8-iso-PGF-$_{2\alpha}$ (pg/mg creatinine) According to Demographic Characteristics Among Men with COPD in the Double Placebo Arm (n=23) of the RAS to SELECT[a]

Characteristic	Urine 8-iso-PGF-$_{2\alpha}$	
	Mean (SD)	Median (Range)
Full Sample	449.5 (407.9)	330.2 (81.9-1677.9)
Age		
50-59	450.0 (369.9)	450.0 (188.4-711.6)
60-69	419.4 (408.0)	226.4 (81.9-1249.3)
≥70	476.7 (448.1)	364.6 (113.3-1677.9)
Ethnicity/Race		
African-American	369.3 (294.2)	196.4 (125.8-985.0)
Caucasian/ Other	501.0 (470.1)	365.4 (81.9-1677.9)
Smoking Status[b]		
Never	166.9 (69.9)	150.8 (109.3-256.4)
Former	299.6 (210.2)	247.3 (81.9-830.0)
Current smokers		
<5 cigarettes/day	*	*
≥5 cigarettes/day	809.1 (526.5)	633.1 (160.4-1677.9)
Body Mass Index, kg/m^2		
≤ 25	458.1 (370.0)	380.0 (81.9-1249.3)
≥25	440.1 463.9	247.3 (113.3-1677.9)

[a] RAS, Respiratory Ancillary to SELECT; SELECT, The Selenium and Vitamin E Cancer Prevention Trial; COPD, Chronic Obstructive Pulmonary Disease

[b] Smoking dose given for current smokers only

* ≤ 1 participant in this category

Supplemental Table 4 Linear Regression Estimates of the Effect of Treatment on Urine 8-iso-PGF-$_{2\alpha}$ in Men with COPD in the RAS[a]

	Placebo	Vitamin E	Selenium	Vitamin E + Selenium
Model-derived Estimates:				
Predicted urine 8-iso-PGF-$_{2\alpha}$, pg/mg creatinine[b]	324	277	302	258
% change in urine 8-iso-PGF-$_{2\alpha}$[c]	Reference group	-15%	-7%	-21%
Model Results:				
Regression Coefficient[d]	Reference group	-0.156	-0.064	-0.228
SE[e]	-	0.195	0.189	0.192
p-value[f]	-	0.4268	0.7352	0.2381

[a] RAS, Respiratory Ancillary to SELECT; SELECT, The Selenium and Vitamin E Cancer Prevention Trial; Chronic Obstructive Pulmonary Disease (COPD) cases, N=107, includes all men with self-reported physician-diagnosed emphysema, chronic bronchitis and/or COPD; least squares regression included treatment variables and adjusted for age, race, and smoking status; Type III P-value for set of treatment variables, 0.6357

[b] ln(geometric mean)

[c] Percent (%) change in urine 8-iso-PGF-$_{2\alpha}$ concentration; values at 36 months in each treatment group compared to placebo/placebo reference group

[d] Regression coefficient conveys estimated difference in ln urine 8-iso-PGF-$_{2\alpha}$ concentration for each treatment group compared to placebo

[e] SE, standard error of regression coefficient

[f] P-value, Wald's test of regression coefficient

Supplemental Table 5 Linear Regression to Estimate the Effect of Treatment (vitamin E and combined vitamin E + selenium arms combined) on Urine 8-iso-PGF-2α in Current Cigarette Smokers and COPD Cases in the RAS to SELECT[a]

	Placebo	Any Vitamin E
Current Cigarette Smokers[b]		
Model-derived Estimates:		
Predicted[c] urine 8-iso-PGF-$_{2\alpha}$, pg/mg creatinine	379	320
% change in urine 8-iso-PGF-$_{2\alpha}$[d]	Reference Group	-16%
Model Results:		
Regression Coefficient[e]	Reference Group	-0.160
SE[f]	-	0.087
p-value[g]	-	0.0669
COPD Cases[h]		
Model-derived Estimates:		
Predicted urine 8-iso-PGF-$_{2\alpha}$, pg/mg creatinine[c]	316	255
% change in urine 8-iso-PGF-$_{2\alpha}$[d]	Reference group	-19%
Model Results:		
Regression Coefficient[e]	Reference group	-0.198
SE[f]	-	0.172
p-value[g]	-	0.2544

[a] RAS, Respiratory Ancillary to SELECT; SELECT, The Selenium and Vitamin E Cancer Prevention Trial; COPD, Chronic Obstructive Pulmonary Disease

[b] Current cigarette smokers, N=246, excluding COPD cases; least squares regression included treatment variables and adjusted for age and race

[c] ln(geometric mean)

[d] Percent (%) change in urine 8-iso-PGF-$_{2\alpha}$ concentration; each treatment group compared to placebo/placebo reference group

[e] Regression coefficient conveys estimated difference in ln urine 8-iso-PGF-$_{2\alpha}$ concentration for each treatment group compared to placebo

[f] SE, standard error of regression coefficient
[g] P-value, Wald's test of regression coefficient
[h] COPD cases, N=76, includes all men with self-reported physician-diagnosed emphysema, chronic bronchitis and/or COPD; least squares regression included treatment variables and adjusted for age, race, and smoking status

Supplemental Table 6 Association of Lung Function (FEV_1) with urine 8-iso-$PGF_{2\alpha}$ Estimated in Hierarchical Linear Regression Models for Men with COPD in the RAS to SELECT[a]

		Vitamin E	Selenium	Vitamin E + Selenium
Model-derived Estimates of Treatment[b] x Time Interaction[c]:				
Unadjusted	Regression Coefficient[d]	2.7	7.4	-43.1
	SE[e]	24.6	23.3	24.9
	P-value[f]	0.9120	0.7509	0.0880
Adjusted[g,h]	Regression Coefficient[d]	9.6	9.6	-29.3
	SE[e]	26.6	24.6	27.1
	P-value[f]	0.7184	0.6980	0.2819

[a]FEV1, forced expiratory volume in the first second; COPD, Chronic Obstructive Pulmonary Disease; RAS, Respiratory Ancillary to SELECT; SELECT, The Selenium and Vitamin E Cancer Prevention Trial;
COPD cases, N=105, includes all men with self-reported physician-diagnosed emphysema, chronic bronchitis and/or COPD; regression models included treatment variables and adjusted for age, race, and smoking status; Type III P-value for set of treatment variables, unadjusted P= 0.0870, adjusted P=0.3049
[b] Each treatment arm is compared to placebo/placebo, the reference group;
[c] Time (years), is the time variable in the mixed model; the coefficient for time conveys the average annual change in the lung function; the interaction of time * treatment conveys the effect of treatment on annual decline in lung function
[d] Regression coefficient conveys estimated difference in rate of decline in lung function for each treatment group compared to placebo. Model included age, height, race, smoking status (for COPD subset), time (years), treatment, F2-IsoPs, and 2-way interaction terms between time and smoking status, treatment, and F2-IsoPs. Urine F2-IsoP was ln-transformed.
[e]SE, standard error of regression coefficient
[f] P-value, Wald's test of regression coefficient
[g] Adjusted model accounts for F2-IsoPs and interaction between time and F2-IsoPs
[h] lnF2-IsoPs β=-182.0 (125.6), P=0.1497; lnF2-IsoPs x time β=21.2 (14.1), P=0.1359; mean rate of decline in men with COPD was -182.2 mL/yr

Supplemental Table 7 Evidence for Mediation of Treatment Effects on FEF_{25-75} by Urine 8-iso-$PGF_{2\alpha}$; Estimated in Hierarchical Linear Regression Models for Men in the Respiratory Ancillary Study to SELECT[a]

		Vitamin E	Selenium	Vitamin E + Selenium
Current Cigarette Smokers[b]				
Model-derived Estimates of Treatment[c] x Time Interaction[d]:				
Unadjusted	Regression Coefficient[e]	24.9	18.0	32.4
	SE[f]	35.5	39.7	36.8
	P-value[g]	0.4831	0.6504	0.3787
Adjusted[h,i]	Regression Coefficient[e]	36.8	11.0	42.1
	SE[f]	35.8	39.8	37.0
	P-value[g]	0.3037	0.7827	0.2562
COPD Cases[j]				
Model-derived Estimates of Treatment[c] x Time Interaction[d]:				
Unadjusted	Regression Coefficient[e]	-21.6	-8.0	-74.6
	SE[f]	54.1	49.2	52.7
	P-value[g]	0.6903	0.8718	0.1590
Adjusted[h,k]	Regression Coefficient[e]	-14.1	-6.9	-62.7
	SE[f]	55.1	49.2	54.7
	p-value[g]	0.7989	0.8885	0.2539

[a] FEF_{25-75}, forced expiratory flow rate between the 25th and 75th percentile of the forced vital capacity (FVC); RAS, Respiratory Ancillary to SELECT; SELECT, The Selenium and Vitamin E Cancer Prevention Trial;

[b] Current cigarette smokers, N=302, excluding cases of chronic obstructive pulmonary disease (COPD); least squares regression included treatment variables and adjusted for age and race; Type III P-value for set of treatment variables, P=0.8370 in unadjusted model, P=0.6316 in adjusted model

[c] Each treatment arm is compared to placebo/placebo, the reference group;

[d] Time, years is the time variable in the mixed model; the coefficient for time conveys the average annual change in the lung function outcome; the interaction of time * treatment indicates the effect of treatment on annual decline in the lung function outcome

[e] Regression coefficient conveys estimated difference in rate of decline in lung function for each treatment group compared to placebo. Model included age, height, race, smoking status (for COPD subset), time (years), treatment, F2-IsoPs, and 2-way interaction terms between time and smoking status, treatment, and F2-IsoPs. Urine F2-IsoPs was ln-transformed.

[f] SE, standard error of regression coefficient

[g] P-value, Wald's test of regression coefficient

[h] Adjusted model further accounts for F2-IsoPs and the time by F2-IsoP interaction

[i] lnF2-IsoPs β=-255.2 (93.0), P=0.0063; lnF2-IsoPs x time β=41.8 (20.1), P=0.0383; mean rate of decline 317.9 (122.3)mL/sec/yr

[j] COPD cases, N=104, includes all men with self-reported physician-diagnosed emphysema, chronic bronchitis and/or COPD; least squares regression included treatment variables and adjusted for age, race, and smoking status; Type III P-value for set of treatment variables, P=0.5941

[k] lnF2-IsoPs β=-186.1 (162.6), P=0.2542; lnF2-IsoPs x time β=27.6 (29.0), P=0.3438; mean rate of decline 247.78mL/sec/yr

Supplemental Table 8 Effects of Treatment on Rate of Decline in FEV_1 in the RAS to SELECT[a]: Mediation of Effects by Urine 8-iso-$PGF_{2\alpha}$

	Current Smokers (N=310)			COPD cases (N=105)		
Variable	β[b]	SE	P-value	β[b]	SE	P-value
Age, years	-44.70	5.23	<0.0001	-30.97	10.91	0.0055
Height, cm	21.86	4.01	<0.0001	21.61	11.38	0.0607
Race						
African American	-390.23	65.31	<0.0001	-167.65	161.23	0.3010
Smoking Status						
Current Smoker	-	-	-	-619.50	296.42	0.0387
Former Smoker	-	-	-	-805.86	284.59	0.0054
Time, y[c]	-37.46	52.08	0.4728	-182.21	80.84	0.0257
Time by Smoking Status						
Current Smoker	-	-	-	26.41	30.58	0.3892
Former Smoker	-	-	-	42.66	28.44	0.1359
Treatment[d]						
Vitamin E	-244.14	92.67	0.0087	201.06	244.03	0.4116
Selenium	-81.23	98.22	0.4087	68.74	232.20	0.7677
Vitamin E + Selenium	-191.73	91.95	0.0376	500.11	237.61	0.0374
Time by Treatment						
Vitamin E	22.03	15.46	0.1555	9.60	26.58	0.7184
Selenium	1.00	16.70	0.9525	9.58	24.63	0.6980
Vitamin E + Selenium	14.02	15.42	0.3641	-29.32	27.14	0.2819
8-iso-$PGF_{2\alpha}$[e]	-94.96	51.88	0.0679	-182.03	125.55	0.1497
Time by 8-iso-$PGF_{2\alpha}$[d,e]	-2.29	8.69	0.7922	21.18	14.12	0.1359

[a] RAS, Respiratory Ancillary to SELECT; SELECT, The Selenium and Vitamin E Cancer Prevention Trial; COPD, Chronic Obstructive Pulmonary Disease

[b] β is the regression coefficient from hierarchical linear regression models, adjusted for all variables shown in table;

[c] Time, years is the time variable in the mixed model; the coefficient for time conveys the average annual change in the lung function outcome; the interaction of time * treatment indicates the effect of treatment on annual decline in the lung function outcome

[d] Each treatment arm is compared to placebo/placebo, the reference group;

[e] Urine 8-iso-$PGF_{2\alpha}$ was ln-transformed

CHAPTER 3

THE RESPONSE TO VITAMIN E SUPPLEMENTATION

IN THE RESPIRATORY ANCILLARY STUDY TO SELECT

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Running Head: Plasma Response to Vitamin E Supplementation

Keywords: alpha-tocopherol, gamma-tocopherol, antioxidant nutrients, randomized controlled trial, vitamins/administration & dosage

Abstract

Background Vitamin E has antioxidant properties, which may promote beneficial health outcomes. Plasma vitamin E levels vary between individuals, but to a lesser extent within an individual over time, and there is greater variation in supplement users compared to non-users, indicating variability in response to supplemental vitamin E. Little is known about individual characteristics that affect the degree to which plasma concentrations increase in response to supplementation.

Methods We investigated the association between participant characteristics and change in plasma concentration in response to vitamin E supplementation (400 mg/day *all rac* α-tocopheryl acetate) over three years in participants of the Respiratory Ancillary Study (RAS) to the Selenium and Vitamin E Cancer Prevention Trial (SELECT).

Results On average, supplementation increased plasma α-tocopherol and decreased plasma γ-tocopherol. Race, however, affected the magnitude of the change in plasma tocopherol, for both α- and γ-tocopherol. African American men had lower baseline plasma α-tocopherol, and had lower increases in α-tocopherol in response to supplementation (about 50% lower) in comparison to European Americans ($p<0.0001$). All associations were independent of cholesterol and adjustment for other covariates.

Conclusions Future studies should consider the magnitude of the change in plasma concentration in relation to the effects of supplementation on health endpoints in order to fully assess the effects of supplementation. The apparently lower response among African Americans may either indicate less possible benefit, or may indicate more immediate use of supplementation, thus more possible benefit. The identification of subgroups that have

differential responses to supplementation, such as African Americans, may assist in understanding intervention effects or in the development of alternate therapies.

Introduction

Vitamin E, an essential lipid-soluble nutrient, is the most abundant antioxidant agent in human plasma. Its primary role in the body is to maintain the integrity of cell membranes by eliminating free radicals and thus preventing cellular damage caused by reactive oxygen species (ROS)(1). Vitamin E is comprised of four tocopherols and four tocotrienols, which are differentiated by the structure of the chromanol head and the phytyl tail. The predominant form of vitamin E in the Western diet is gamma(γ)-tocopherol, which is primarily found in plant sources such as vegetable oils and spreads, grains, nuts, fruits and vegetables(1). In contrast, the most abundant form in blood is alpha(α)-tocopherol due to preferential binding of the α form by the α-tocopherol transfer protein (α-TTP) in the liver(2). Oxidative stress is hypothesized to play a key role in the pathophysiology of chronic diseases, thus supplementation with vitamin E, a chain-breaking antioxidant, has long been of interest as a preventive and/or a treatment(3).

The hypothesized beneficial effects of vitamin E on health endpoints have not been borne out in published clinical trials of cardiovascular and cancer endpoints(4, 5). However, recent studies of lung outcomes(6, 7) support a protective effect of vitamin E supplementation, particularly in cigarette smokers. Given that the proposed effects of vitamin E are mediated by transport of vitamin E through the circulation to the relevant target tissue, understanding inter-individual differences in change in plasma vitamin E in response to supplementation is important to understand the potential to benefit from vitamin E supplementation.

The National Health and Nutrition Examination Survey (NHANES III) identified greater variability in plasma α-tocopherol status in individuals who consume vitamin E

supplements compared to non-users of supplement(8), confirming inter-individual variability in response to supplementation. During vitamin E supplementation, the within-individual response (where response is change in plasma concentration) is highly repeatable; in contrast, there is far greater variation between individuals in response to supplementation (9-12). Although such differences are undoubtedly affected by the form and dose of vitamin E supplement used, there are few studies that address the factors that contribute to differences between individuals in response to supplementation with vitamin E. A few previous studies investigated the kinetics(9, 13-17) and inter-individual variability of vitamin E response(11), but these studies were limited by small sample size, short duration, and/or failure to consider cholesterol changes during the supplemented period.

Alpha-tocopherol concentration in both plasma and adipose tissue is highly variable between individuals(18-20), and past cross-sectional studies identified several factors associated with plasma α-tocopherol concentration. Age was positively associated with plasma α-tocopherol concentration, but effect sizes were small and associations were attenuated in models adjusted for cholesterol(18, 19, 21-24). An increased use of supplements with increasing age may also explain the weak association of age with plasma vitamin E (8). Putative associations with age underscore the importance of accounting for plasma lipids since vitamin E is transported by plasma lipoproteins, and more of the transport molecule is associated with higher plasma vitamin E concentration (19, 23, 25).

African Americans have lower plasma α-tocopherol concentration compared to European Americans (18, 18, 19, 23), but the lower prevalence of supplement use in African-Americans may partly explain these differences(8). On average, African Americans also consume lower amounts of dietary vitamin E(18), which is expected to contribute to

lower plasma concentrations in comparison to other race/ethnicity groups(18). The basis for reported differences by race/ethnicity has not been fully explored in existing literature (18, 18, 19, 23).

Plasma vitamin E concentration is lower in cigarette smokers(26, 27), which may be due to higher oxidative stress in smokers leading to depletion of plasma vitamin E. In support of this theory, among individuals dosed with labeled α-tocopherol, the α-tocopherol disappearance rate was faster in smokers compared to non-smokers(28, 29). Other studies, however, report no significant differences in plasma α-tocopherol concentration between smokers and non-smokers(23, 25, 30-33), thus findings are mixed.

Body mass index (BMI), a proxy for body adiposity, is associated with lower plasma α-tocopherol in most studies, although not all studies adjust for plasma lipids (18, 23, 25, 34-39). Since obese individuals may also have greater levels of oxidative stress (40), lower α-tocopherol levels in obese individuals may be the end result of greater utilization of α-tocopherol to quench oxygen radicals. All prior studies are cross-sectional, which is a significant limitation in making a causal inference.

Sequence variation in genes involved in the absorption, transport, and metabolism of vitamin E is associated with plasma α-tocopherol concentration (41-44), and variants in vitamin E-related genes are proposed to account, in part, for differential effects of vitamin E supplementation on disease endpoints(45). To date, a single study reported genetic variants were associated with plasma response to vitamin E supplementation(46), but the sample was comprised entirely of heavy-smoking Finnish males, limiting generalizability.

Understanding predictors of response to vitamin E supplementation will improve the interpretation of existing clinical trials, contribute to improved study designs in future

investigations of the role of vitamin E in health, and will help to elucidate the true effects of supplementation on health endpoints. Furthermore, if vitamin E does prevent and/or mitigate progression of some diseases, the identification of groups who do not respond to vitamin E will inform the development of alternative approaches. The study reported herein investigated the variation in plasma change in response to supplementation with vitamin E, using data from a large, randomized, placebo-controlled trial of vitamin E and selenium supplementation; the study outcome was the change in plasma α-tocopherol concentration from the study baseline to thirty-six months on supplementation.

Methods

Study Population

The Selenium and Vitamin E Cancer Prevention Trial (SELECT) randomized 35,433 men aged fifty and older at 428 study sites in North America to 400 IU/day *all rac*-α-tocopherol (α-tocopheryl acetate, hereafter denoted as vitamin E) + selenium placebo, vitamin E placebo + 200 μg/day selenomethionine (L-selenomethionine, hereafter denoted as selenium), vitamin E + selenium, or, vitamin E placebo + selenium placebo. The Respiratory Ancillary Study (RAS) to SELECT registered a subset of 2,920 men to investigate the effect of treatment on lung function, and purposefully oversampled cigarette smokers due to their higher risk of steep decline lung function(6). All participants in the RAS in either vitamin E arm (n=588 vitamin E + selenium, n=578 vitamin E + selenium placebo) with plasma samples at both baseline and year three, were studied. In addition, paired plasma samples from 100 men in the placebo arm were randomly selected as a comparison group.

Vitamin E Assay

Blood samples were collected at SELECT baseline and year 3 on study, and stored in -80° C freezers for a maximum of nine years. Tocopherol concentrations in plasma samples were determined by gas chromatography-mass spectrometry (GC-MS) using a Hewlett Packard 6890 gas chromatograph coupled to a Hewlett Packard 6890 mass spectrometer. Tocopherol and free cholesterol (as silyl ethers) were quantitated against the d9-α-tocopherol internal standard, adjusting the cholesterol values for pre-determined differences in detector response. While most studies assess total cholesterol, GC-MS assesses free unesterified cholesterol; hereafter, we use cholesterol-adjusted tocopherol to refer to free unesterified cholesterol adjustments. Adjustment for free cholesterol serves the same function as adjusting for total cholesterol, however, given the strong correlation between total and free cholesterol(47); additionally, the proportion of free to total cholesterol is considered to be relatively constant regardless of changes in total cholesterol(47, 48). All samples were assayed over a six-month period by a single technician blinded to sample identity and randomization arm. Plasma samples were assayed in batches of eight samples (four paired samples; baseline and year three sample for an individual assayed in same run) with one set of duplicates per batch. A total of 321 duplicate pairs were assayed and the average coefficient of variation (CV), reflecting within-batch variation, was calculated;the CV was 9.6% for total α-tocopherol and 1.7% for free cholesterol-adjusted α-tocopherol; the CV for γ-tocopherol was 13.4%. In addition, on each assay day a control sample was assayed in the first and last run of the day. These samples provided data on between-run variability; the standard deviation was 6.5 μmol/L and 2.1 μmol α-toc/mmol cholesterol for α-tocopherol and cholesterol-adjusted α-

tocopherol, respectively. Coefficients of variation for all control samples were 13.9% and 12.9%, for α-tocopherol and cholesterol-adjusted α-tocopherol, respectively.

In response to supplementation, plasma α -tocopherol concentration increases and then reaches a plateau by about day 4-5 on vitamin E supplement(12); thereafter, plasma concentration remains at about the same level with continued use of supplement. SELECT protocols required men to stop using supplements prior to randomization, but did not define a formal 'washout' period prior to randomization. Plasma concentration of α-tocopherol in supplemented individuals is estimated to return to the un-supplemented value between 12 to 20 days after withdrawal of supplement(12). In SELECT, the time between the last use of a vitamin E supplement (pre-randomization) and study randomization is unknown. Including individuals whose baseline plasma vitamin E concentration was affected by recent use of supplements invalidates the calculation of change in plasma concentration in response to study intervention, thus criteria were developed to identify and exclude participants with strong evidence of supplement use that affected the baseline plasma vitamin E concentration; these criteria were independent of any consideration of outcome data. Meeting all four of the following criteria identified baseline blood samples whose plasma vitamin E was affected by recent supplement use: self-reported use, in the past year, of multivitamin or supplements containing $\geq$ 50 IU/d of α-tocopherol; plasma γ-tocopherol concentration at baseline ≤2 μmol/L; >5% increase in plasma γ-tocopherol concentration from baseline to year 3; and presence of a supplement use-associated peak on GC-MS in baseline sample (prior work in an unrelated, small randomized trial identified this GC-MS peak *only* in supplement users(49). Together the four criteria were evidence that the gap between prior use of non-study supplement and

randomization was not long enough to achieve a true, un-supplemented baseline plasma vitamin E concentration; 78 participants were excluded for this reason.

Statistical Analysis

All analyses were carried out in SAS, version 9.3 (SAS Institute, Cary, NC). α-tocopherol and γ-tocopherol levels, age, smoking dose, years smoked, and cholesterol were continuous variables. Race and smoking status were categorical variables. BMI was considered as both a categorical variable (obese, overweight compared to normal weight) and a continuous variable in separate models. Selenium supplementation in the combination treatment arm did not have a significant effect on plasma vitamin E concentrations; therefore, all participants taking vitamin E supplements (participants in the vitamin E plus placebo and in the vitamin E plus selenium arms) were combined into one group for analyses. Regression models estimated the association of participant characteristics with the change in plasma tocopherol concentration, where tocopherol change was calculated as the year three minus the baseline value; change in α- and γ-tocopherol were considered in separate models. Primary findings are based on tocopherol adjusted for free cholesterol at the corresponding time point, but models unadjusted for cholesterol were also considered to provide comparisons to published studies. One participant was excluded from analyses due to assay failure for the thirty-six month blood sample.

Different variance-covariance matrices were investigated for the full RAS pulmonary function dataset, and variance components was selected as the best matrix choice based on comparison of AIC criteria and consideration of degrees of freedom. The Kenward-Rodgers method for standard error and denominator degrees-of-freedom (ddfm)

correction was selected (ddfm=kr) for computing the denominator degrees of freedom for the tests of fixed effects. No discernable pattern was detected in plots of studentized residuals versus predicted values, and there was no difference in residuals by treatment arm. In a sensitivity analysis including outlying observations, there was no difference in parameter estimates compared to those from the edited dataset which excluded outliers. Refer to the Appendix for further details.

Results

Participant Characteristics

At baseline, participant characteristics were evenly balanced by arm (Table 1; placebo group in Supplemental Table 1), and the mean age was 63 years. The RAS selectively registered cigarette smokers, thus 16% of men were current smokers and 51% were former smokers. The average smoking dose in current smokers was 18.3 (SD 10.9) cigarettes per day. The mean years smoked was 21.1 (SD 12.9) in former smokers and 37.8 (SD 12.7) in current smokers. African Americans comprised 23% of the sample, and cigarette smoking was more prevalent in this group (29% current smokers, 46% former) compared to European Americans (12% current, 53% former). The majority of the sample was overweight or obese, accounting for 46% and 34% of the sample, respectively, and the mean BMI was 28.9 (SD 4.7).

At study baseline, prior to randomization, plasma concentrations of α- and γ-tocopherol, free cholesterol, and the ratio of α- to γ-tocopherol varied (Table 1). As expected, the variability in plasma α-tocopherol was reduced after adjusting cholesterol, but a 5- to 6-fold difference from the top to bottom of the range persisted (compared to

about a ten-fold difference in unadjusted values). At baseline, mean α- and γ-tocopherol values were 19.6 and 3.6 μmol/L, respectively.

Association of Participant Characteristics with Plasma Vitamin E

At the study baseline, prior to supplementation, plasma tocopherol concentrations differed by race and smoking status (Supplemental Table 3). African Americans had lower α-tocopherol concentrations compared to European Americans (8.1 ± 2.9 (mean ± SD) and 9.2 ± 3.0 μmol/mmol free cholesterol, respectively), and higher γ-tocopherol concentrations (1.9 ± 0.9 and 1.4 ± 0.8 μmol/mmol free cholesterol, respectively). Former and never smokers had similar α-tocopherol concentrations at baseline (both 9.1 μmol/mmol free cholesterol), and current smokers had slightly lower concentrations (8.0 μmol/mmol free cholesterol). There was little or no association of BMI with plasma α-tocopherol concentrations at baseline (overweight 8.9, obese 9.0, normal weight 8.9 μmol/mmol free cholesterol).

Using the clinical definition of vitamin E deficiency (11.6 μmol/L), 4.5% of RAS participants were vitamin E deficient at baseline (data not shown). The prevalence of vitamin E deficiency varied by race such that 9% of African Americans were vitamin E deficient at baseline, compared to 3% of European Americans.

Given the similarity of baseline characteristics (Table 1) between the two vitamin E arms (vitamin E + selenium and vitamin E + placebo arms), and in light of similar mean plasma α-tocopherol at year three, the two groups were combined for all further analyses. Hereafter, vitamin E refers to men on any vitamin E, either alone or combined with selenium.

Plasma α-tocopherol concentration increased by 40% from baseline to year three in participants randomized to active vitamin E supplement (Table 2); plasma concentrations decreased about 13% in the small sample of participants assayed in the placebo arm (Supplemental Table 2). Differences in cholesterol did not account for the inter-individual variation in change in plasma α-tocopherol concentration in response to supplementation. In men on vitamin E, γ-tocopherol levels decreased by about 50% (Table 2), compared to an increase of 11% in the placebo arm (Supplemental Table 2). The ratio of α-tocopherol to γ-tocopherol increased more than three-fold in the vitamin E arms (Table 2), but there was little or no change in the placebo arm (Supplemental Table 2). Given that vitamin E is a lipid-soluble vitamin, free cholesterol-adjusted results are the focus of the further analyses.

Factors Associated with Change in Plasma Alpha-Tocopherol

Higher plasma concentrations of α-tocopherol at baseline were associated with less increase in plasma α-tocopherol in response to supplementation, and higher baseline γ-tocopherol was associated with greater change in α-tocopherol (Tables 3, 5; $p<0.0001$). Increasing age was associated with greater change in plasma α-tocopherol in models unadjusted for cholesterol (data not shown), but the association was attenuated when adjusted for cholesterol. There was an association of BMI with change such that individuals with greater BMI had a greater change in plasma α-tocopherol ($p=0.0479$), and this association remained after cholesterol adjustment (Tables 3, 5).

Current smokers had less increase in plasma α-tocopherol concentration compared to never smokers (Type III P-value=0.1000), but in cholesterol-adjusted models differences by smoking status were attenuated (Type III P-value=0.3546), particularly after adjusting for other smoking variables such as smoking dose and years smoked (Tables 3, 5). The

baseline variables smoking dose (cigarettes per day, p=0.6125) and years smoked (p=0.1577) had little or no association with change in plasma α-tocopherol concentration in response to supplementation.

African Americans had lower plasma α-tocopherol concentrations, by about 1 μmol/mmol free cholesterol, at the study baseline (Supplemental Table 3), and had less of an increase in plasma α-tocopherol concentration in response to supplementation in unadjusted models (β=-1.33, P=<0.0001, Supplemental Table 5), even after adjusting for lower plasma α-tocopherol concentration at baseline. In fully adjusted models, the lower response to supplementation in African Americans remained, and adjusting for other variables did not attenuate the effect (β=-1.26, P=<0.0001, Table 5). Thus, European Americans had both higher baseline plasma α-tocopherol concentration and a greater increase in plasma α-tocopherol in response to supplementation, regardless of consideration of potentially confounding variables (Tables 3, 5). In models investigating change in plasma α-tocopherol concentrations, there was no interaction between race and baseline plasma α-tocopherol concentration (data not shown). Considering all predictors, in fully adjusted models which explained 8.1% of the variability, race was the strongest predictor of response to supplementation, explaining 1.4% of the variability, followed by baseline plasma α-tocopherol concentration (β=-0.36, P<0.0001; R^2 = 6.7%) and BMI (β=0.05, P<0.0479; R^2 = 0.3%) (Table 5).

Factors Associated with Change in Plasma Gamma-Tocopherol

Lower baseline plasma γ-tocopherol concentration is associated with less change in γ-tocopherol concentration with supplementation (Tables 4, 5; p<0.0001); supplementation with α-tocopherol reduces plasma γ-tocopherol levels, and the potential

for further γ-tocopherol-lowering may be less as baseline γ-tocopherol approaches zero. About 84% of participants supplemented with vitamin E experienced a decrease in plasma γ-tocopherol concentration, but the decrease was of greater magnitude in men with lower baseline plasma α-tocopherol concentration; this association persisted in models also adjusted for baseline γ-tocopherol concentration.

In comparison to European Americans, African Americans have higher baseline plasma γ-tocopherol concentrations (Supplemental Table 3) and less decrease in plasma γ-tocopherol concentration (β=0.2569, $p<0.0001$; Supplemental Table 5) with supplementation (35% smaller decrease; Table 4); these differences attenuated slightly, but remained highly statistically significant after adjusting for covariates (Table 5). In the fully adjusted model which explained 56% of the variability (Table 5), baseline γ-tocopherol (β=-0.73, $P<0.0001$; R^2=54%) and race (β=-0.24, $P<0.0001$; R^2= 1.4%) are predictive of change in γ-tocopherol.

Discussion

Race was associated with the change in plasma tocopherol concentration in men supplemented with vitamin E, specifically 400 IU/d *all rac*-α-tocopheryl acetate. African American participants had a lesser increase in plasma α-tocopherol concentration in response to supplementation compared to European Americans, and the association was independent of change in cholesterol and other covariates.

Consistent with prior reports, African Americans had lower plasma α-tocopherol concentration at the study baseline, prior to supplementation, and this could not be explained by differential supplement use by race given baseline bloods are mainly

representative of un-supplemented status. Although SELECT did not have a formal 'washout' period, the analysis reported herein used a set of criteria to objectively exclude participants whose baseline plasma samples were likely to be affected by recent supplement use. Lower plasma α-tocopherol concentration in African Americans may be explained by variation in genes involved in vitamin E absorption, transport, or metabolism; a recent GWAS identified SNPs associated with α-tocopherol levels after vitamin E supplementation(46). Alternatively, African-Americans may have lower plasma α-tocopherol concentrations due to differences in environmental exposures and/or in metabolic demands that affect the disposition and utilization of systemic antioxidants.

Response to nutritional supplementation is expected to vary by participant characteristics, including baseline nutriture, thus comparison of baseline nutriture in the current study provides information about generalizability of these findings to the United States population. The median plasma α-tocopherol value in the RAS (19.6 μmol/L), was similar to findings reported for males in NHANES III who did not use supplements containing vitamin E (22.85 μmol/L) (18). The comparability between the baseline α-tocopherol concentration in the RAS and the NHANES value from un-supplemented males confirms that at baseline, participants in RAS were similar to the US Population.

Gamma-tocopherol concentrations and the ratio of α- to γ-tocopherol differed between the RAS and the NHANES III data (18). The baseline plasma concentration of γ-tocopherol in RAS participants was 3.6 μmol/L, which was about half the value in NHANES III male non-users of supplement (6.01 μmol/L) (18); similarly the mean α-to-γ-tocopherol ratio in RAS participants (~11) was about three times higher than the mean reported for male non-users of supplements in NHANES III (3.61) (18). These discrepancies may be due to

differences in sample characteristics, including race distributions given that the proportion of African-Americans is much higher in RAS in comparison to NHANES III (separate values by race are not reported for NHANES III). The differences are unlikely to be attributed to supplement use in the RAS because we excluded participants with baseline plasma that may have been affected by recent supplement use. Gamma-tocopherol is understudied, and the biokinetics are less well characterized in comparison to α-tocopherol. Race-specific data are needed to reach a deeper understanding of the identified distribution differences in the RAS in comparison to NHANES III.

Change in Plasma Tocopherols

The most striking finding is that race is a meaningful predictor of change in α-tocopherol; specifically, African Americans had less increase in plasma concentrations in response to supplementation in comparison to European Americans. While plasma α-tocopherol levels did not increase as much in African Americans, we cannot necessarily conclude that African Americans do not benefit from supplementation in terms of improved health outcomes. Less change, and lower mean concentration achieved by year three, may indicate that a greater proportion of the supplemental vitamin E is used, leading to lower circulating vitamin E; consistent with this hypothesis, previous studies reported decreased α-tocopherol levels in smokers(26, 27), who have greater oxidative stress levels(50, 51). While a greater proportion of African Americans (compared to European Americans) were smokers, even among never smokers African Americans had higher levels of a biomarker of systemic oxidative stress, urine F_2-isoprostanes(52). A recently developed method to detect oxidative urinary α-tocopherol metabolites(53) may offer a useful tool to provide evidence about whether vitamin E is utilized to a greater extent in

African Americans. However, this method is new and requires replication and validation before implementation in a large-scale epidemiologic study. Given that African Americans have higher levels of oxidative stress(52), however, it is plausible that less change in response to supplements in African Americans signifies greater 'use' of the nutrient.

Smoking status was also predictive of change in plasma α-tocopherol concentration, and although associations were attenuated in the fully adjusted model, these models may be over-adjusted. Less change in plasma α-tocopherol among current smokers is consistent with previous findings that smokers have faster disappearance of vitamin E from their plasma(28, 29), and achieve lower plasma α-tocopherol levels via supplementation compared to non-smokers (54). While our results do not agree with a small biokinetic study that indicated no effect of smoking status on plasma response to vitamin E supplementation(55), our study is a much larger, more diverse study of long-term supplementation which includes African Americans, allowing for a stronger test of hypothesis.

As expected, predictors of α-tocopherol were also associated with γ-tocopherol. While vitamin E supplementation led to a decrease in γ-tocopherol in both African Americans and non-African Americans, there was less change (lower decreases) in African Americans. This finding parallels the finding of less change (lower increases) in α-tocopherol in response to supplementation in African Americans. Since γ-tocopherol and α-tocopherol use the same transfer protein (α-TTP), but α-TTP has a greater affinity for α-tocopherol(2), change in γ-tocopherol is expected to be closely linked with α-tocopherol levels.

The lower increase in plasma α-tocopherol among African Americans on vitamin E is a novel finding. While African Americans generally have a different lipid profile compared to European Americans(56, 57), including lower triglycerides and higher high density lipoprotein (HDL), vitamin E is expected to be proportionally distributed throughout all lipid particles in the plasma. While high levels of some lipids (low-density lipoproteins, triglycerides) are associated with increased risk of disease, we are considering change in plasma concentration of vitamin E independent of any disease state; thus, differential lipid profiles are unlikely to account for the race difference observed. Furthermore, the difference cannot be attributed to lower rates of supplement use among African Americans since all men studied herein were randomized to vitamin E supplements and adherence to study supplement was high, with no evidence of differential adherence by race/ethnicity.

Differences in plasma response may be partly attributable to genetic variation(58, 59); two non-synonymous SNPs in the *CYP4F2* gene (rs3093105, W12G; rs2108622,V433M), which catalyzes the first step in the vitamin E-ω-oxidation pathway alter ω-hydroxylase activity, have different race-specific prevalence. The W12G variant results in greater ω-hydroxylase activity(59) and, given the higher prevalence of this variant among African Americans(60), may contribute to a lower plasma response to supplementation seen among African Americans in the RAS. Conversely, the V433M variant, more common among European Americans(60), results in less ω-hydroxylase activity(59) and may therefore be partly responsible for the lower response among African Americans. Future analyses considering genetic variants in the context of human

supplementation trials will better inform whether, and to what extent, racial differences in plasma response to supplementation are attributable to underlying genetic backgrounds.

There are a few limitations, which have been addressed as fully as possible. One limitation is the lack of a traditional 'wash out' period in SELECT, which may affect the accuracy of measuring change in plasma tocopherols. However, biochemical data provide ample support that at baseline, most participant tocopherol concentrations were unaffected by supplement use in the period preceding randomization. Participants in the placebo arm increased in γ-tocopherol slightly from baseline to year three (Supplemental Table 2), indicating that very few men used supplements at baseline, and also indicating that the placebo arm was truly non-supplemented at year three. Plasma assays also confirm that most men in the vitamin E arms at year three were adherent to the study supplement, since measured decreases in plasma γ-tocopherol levels (Table 2) were consistent with expectation for supplement users(61). Dietary data are not considered here, but all participants were randomized to receive the same dosage, duration, and type of vitamin E supplementation, and dietary vitamin E has been shown to have little impact on plasma α-tocopherol levels. Whether response to supplement is modified by dietary intake of vitamin E has not been addressed in this study. Because this study assesses change with two measurements of vitamin E, we are unable to estimate the trajectory of plasma change over time in response to supplementation. Previous studies, however, clearly demonstrate that plasma α-tocopherol levels increase as early as three hours post-supplement(55), with a peak twelve hours after a single oral dose (14). Furthermore, plasma levels plateau by approximately day four in response to daily supplementation with

a consistent dose of vitamin E(14), thus by year three a steady state of plasma α-tocopherol is expected.

While the present study considers a single supplemented level of vitamin E, prior studies demonstrated a linear increase in plasma concentration with supplemental vitamin E (deuterated RRR-α-tocopheryl acetate)(62), with no further increase in plasma levels with supplementation beyond 350 mg/d of RRR α-tocopherol(63). Daily replacement of the vitamin E pool is proposed to explain the plateau in plasma vitamin E at higher levels of supplementation(62).

This study has a strong design, and it addresses important gaps in knowledge by providing new evidence. African Americans are traditionally underrepresented in research, and are not included in many of the published vitamin E trials(64); as a result of intensive recruitment of African Americans by SELECT, African-Americans comprised 23% of the RAS, which allowed for explorations of the effect of race. Most previous studies are cross-sectional, and while vitamin E is proposed to have health benefits that act by transport of vitamin E through the plasma to target tissues, the effect of supplementation on plasma levels is understudied. The GC-MS method allowed for assay of γ-tocopherol in addition to α-tocopherol; while α-tocopherol was previously thought to be the only tocopherol with biological activity, researchers are increasingly interested in the potential effects of γ-tocopherol (65). This is the only study to our knowledge that investigates the effect of long-term α-tocopherol supplementation on change in γ-tocopherol levels, and fully considers the influences of both tocopherols.

Given the need to balance the risks and benefits of supplementation with vitamin E, this study provides critical evidence to deepen understanding of how to identify the subset

of the population most likely to benefit from vitamin E supplementation. If the effect of supplement is hypothesized to act at the target tissue, and thus the nutrient must be transported to the target tissue in plasma lipoproteins, full understanding of plasma change in response to supplementation is needed in order to effectively target nutritional approaches to avoid chronic disease. These data show that African Americans have less response to supplementation with vitamin E. Further data are needed to determine if this reflects a lower functional dose or if delivered doses are being used more quickly in African Americans. The understanding of vitamin E metabolism is still under study, and having better understanding of population-level variation in metabolism is significant in adding to the evidence base for optimal nutrition at the individual level.

Table 1 Baseline Characteristics of Male Participants Randomized to Vitamin E (400 IU/day *all rac*-α-tocopherol) (N=1166) in the Respiratory Ancillary Study to SELECT[a]

		Treatment Arm	
		Vitamin E (n=588)	Vitamin E + Selenium (n=578)
Age (y)		62.6 ± 6.5[b]	62.8 ± 6.4
Ethnicity [n (%)]			
European American		452 (77)	450 (78)
African American		136 (23)	128 (22)
Smoking Status[c] [n(%)]			
Never		190 (32)	188 (33)
Former		299 (51)	299 (52)
Current		95 (16)	90 (16)
Smoking Dose (cigarettes/day)[d]		2.7 ± 7.2	3.1 ± 8.6
Years Smoked[e]		16.9 ± 16.9	16.9 ± 16.8
Body Mass Index (BMI)[f] [n(%)]			
Normal		94 (16)	126 (22)
Overweight		268 (46)	277 (48)
Obese		226 (38)	175 (30)
BMI (kg/m^2)		29.3 ± 4.7	28.5 ± 4.6
Serum Measurements[g]			
α-tocopherol	Unadjusted (μmol/L)	21.3 ± 9.1 19.4 (6.7, 95.9)	21.7 ± 9.3 19.7 (8.3, 79.0)
	Free-cholesterol-adjusted (μmol/mmol chol)	8.9 ± 2.9 8.2 (4.1, 25.8)	9.0 ± 3.2 8.3 (3.6, 29.8)
γ-tocopherol	Unadjusted values (μmol/L)	3.7 ± 2.3 3.2 (0.1, 18.2)	3.5 ± 2.6 2.9 (0.2, 23.4)
	Free-cholesterol-adjusted (μmol/mmol chol)	1.5 ± 0.9 1.4 (0.1, 5.2)	1.4 ± 0.9 1.2 (0.1, 6.2)
α-tocopherol/ γ-tocopherol Ratio		10.3 ± 15.3 5.9 (1.2, 181.2)	11.6 ± 19.1 6.8 (1.0, 232.8)
Free cholesterol (mmol/L)[h]		2465.5 ± 892.1 2302.6 (937, 7764)	2495.6 ± 886.4 2314.6 (1034, 8626)
Vitamin E Deficient[i] [n(%)]		31 (5)	22 (4)

[a] SELECT, The Selenium and Vitamin E Cancer Prevention Trial.

[b] Mean ± SD unless otherwise indicated

[c] 5 participants missing baseline smoking data (by arm: N=1 in E + Se, N=4 in E + placebo)

[d] 6 participants missing smoking dose (by arm: n=1 in E + Se, n=5 in E + placebo); never and former smokers have dose of 0).
[e] 10 participants missing pack-years (by arm: n=3 in E + Se, n=7 in E + placebo); never smokers have value of 0 and contribute to average.
[f] Obese defined as BMI ≥30; overweight defined as BMI 25-30; normal weight, BMI <25, is reference group. This sample did not include underweight individuals.
[g] Mean ± standard deviation; Median (minimum, maximum)
[h] Free cholesterol refers to unesterified lipids
[i] Deficiency defined as <11.6 μmol/L α-tocopherol at baseline

Table 2 Plasma Tocopherol Concentration at Thirty-six Months on Supplement in Male Participants Randomized to Vitamin E (N=1166) in the RAS to SELECT[a]

		Plasma Tocopherol at Study Baseline	Plasma Tocopherol at 36 months	Change in Plasma Tocopherol[b]
α-tocopherol	Unadjusted, μmol/L	21.5 ± 9.2[c]	30.0 ± 13.9	8.5 ± 13.1
		19.5 (6.7, 95.9)[d]	27.3 (7.5, 127.0)	7.1 (-54.5, 108.8)
	Adjusted, μmol/mmol chol	8.9 ± 3.1	12.7 ± 4.6	3.8 ± 4.2
		8.2 (3.6, 29.8)	11.9 (3.3, 47.6)	3.2 (-12.2, 33.9)
γ-tocopherol	Unadjusted, μmol/L	3.6 ± 2.5	1.8 ± 1.6	-1.8 ± 2.4
		2.4 (0.1, 23.4)	1.3 (0.0, 13.4)	-1.5 (-22.2, 9.9)
	Adjusted, μmol/mmol chol	1.5 ± 0.9	0.8 ± 0.6	-0.7 ± 0.9
		1.3 (0.1, 6.2)	0.6 (0.0, 4.9)	-0.6 (-4.6, 2.4)
α-tocopherol/ γ-tocopherol ratio		10.9 ±17.3	34.0 ± 36.6	23.1 ± 36.7
		6.4 (1.0, 232.8)	22.2 (1.2, 290.2)	13.1 (-191.6, 261.7)

[a] Vitamin E includes all participants on vitamin E, 400 IU/day *all rac-α*-tocopherol, which includes vitamin E + placebo and vitamin E + selenium. Placebo refers to participants taking both placebo pills; RAS, Respiratory Ancillary Study; SELECT, The Selenium and Vitamin E Cancer Prevention Trial.

[b] Change in plasma concentration defined as thirty-six month value minus the baseline value.

[c] Mean ± SD, unless otherwise indicated

[d] Median; minimum, maximum in parentheses

Table 3 Model-based Predicted Change in Plasma α-tocopherol (μmol/mmol chol) in Unadjusted and Adjusted Models for Male Participants Randomized to Vitamin E (400 IU/day *all rac-α*-tocopherol) (N=1166) in the RAS to SELECT[a]

Variables in Model:	Unadjusted Model	Adjusted Model[b]
Baseline α-tocopherol[c,d]	3.82	2.58
Baseline γ-tocopherol[d,e]	3.82	2.04
Race		
European American	4.12	2.86
African American	2.79	1.66
Age[f]	3.82	2.58
Smoking Status		
Never	3.97	2.92
Former	3.91	2.44
Current	3.23	2.64
Smoking Dose[d,g]	3.83	2.58
Cumulative Years Smoked[d,h]	3.83	2.58
Body Mass Index (BMI), kg/m^{2}[d,i]	3.82	2.58
BMI[j]		
Normal	3.56	2.38
Overweight	3.78	2.46
Obese	4.03	2.79

[a] Vitamin E includes all participants on vitamin E (including E + placebo and E + selenium); RAS, Respiratory Ancillary Study; SELECT, The Selenium and Vitamin E Cancer Prevention Trial. Change in plasma level defined as thirty-six month value minus the baseline value. All tocopherol values adjusted for free cholesterol at each time point. Unless otherwise noted, models predicting change in α-tocopherol adjust for baseline α-tocopherol level, and models predicting change in γ-tocopherol adjust for baseline γ-tocopherol level.

[b] Adjusted Model includes baseline α-tocopherol, smoking status, smoking dose, years smoked, age, race, and BMI (continuous), unless otherwise noted. For categorical variables, race, smoking status, and BMI category, the mean change in plasma α-tocopherol concentration is predicted (95% confidence interval) for each category.
[c] Mean baseline α-tocopherol level is 8.92 μmol/mmol chol
[d] Values shown for continuous variables are for predicted change in α-tocopherol concentration for the mean of the variable.
[e] Mean baseline plasma γ-tocopherol concentration is 1.5 μmol/mmol chol
[f] Mean age 62.7; 25th percentile 57.9, 75th percentile 66.7
[g] Mean baseline smoking dose 2.9 cigarettes per day (for never smokers and former smokers, dose is zero)
[h] Mean cumulative years smoked 16.87 (for never smokers, years smoked is zero)
[i] Mean BMI 28.9; 25th percentile 25.7, 75th percentile 31.4
[j] Obese defined as BMI ≥30; overweight defined as BMI 25-30; normal weight, BMI <25, serves as reference group. This sample did not include underweight individuals.

Table 4 Model-based Predicted Change in Plasma γ-tocopherol (μmol/mmol chol) in Unadjusted and Adjusted Models for Male Participants Randomized to Vitamin E (400 IU/day *all rac-α*-tocopherol) (N=1166) in the RAS to SELECT[a]

Variables in Model:	Unadjusted Model	Adjusted Model[b]
Baseline α-tocopherol[c,d]	-0.72	-0.46
Baseline γ-tocopherol[d,e]	-0.72	-0.47
Race		
European American	-0.78	-0.53
African American	-0.52	-0.29
Age[f]	-0.72	-0.47
Smoking Status		
Never	-0.74	-0.51
Former	-0.73	-0.46
Current	-0.61	-0.48
Smoking Dose[d,g]	-0.72	-0.48
Cumulative Years Smoked[d,h]	-0.72	-0.48
Body Mass Index (BMI), kg/m^{2}[d,i]	-0.72	-0.47
BMI[j]		
Normal	-0.73	-0.50
Overweight	-0.75	-0.50
Obese	-0.68	-0.44

[a] Vitamin E includes all participants on vitamin E (including vitamin E + placebo and vitamin E + selenium). RAS, Respiratory Ancillary Study; SELECT, The Selenium and Vitamin E Cancer Prevention Trial. Change in plasma concentration defined as thirty-six month value minus the baseline value. All tocopherol values adjusted for free cholesterol at each time point. Unless otherwise noted, models predicting change in γ-tocopherol adjust for baseline γ-tocopherol level. Values represented for categorical variables are for mean predicted level (95% CI).

[b] Model adjusted for all other variables, including baseline γ-tocopherol, smoking status, smoking dose, years smoked, age, race, and BMI.

[c] Mean baseline α-tocopherol level is 8.92 μmol/mmol chol.

[d] Values shown for continuous variables are for predicted change in γ-tocopherol concentration for the mean of the variable.

[e] Mean baseline plasma γ-tocopherol concentration is 1.5 μmol/mmol chol.

[f] Mean age 62.7; 25^{th} percentile 57.9, 75^{th} percentile 66.7

[g] Mean baseline smoking dose 2.9 cigarettes per day (for never smokers and former smokers, dose is zero).

[h] Mean cumulative years smoked 16.87 (for never smokers, years smoked is zero).

[i] Mean BMI 28.9; 25th percentile 25.7, 75th percentile 31.4.
[j] Obese defined as BMI ≥30; overweight defined as BMI 25-30; normal weight, BMI <25, serves as reference group. This sample did not include underweight individuals.

Table 5 Regression Coefficients from Mixed Models (Adjusted Models) for Change in Plasma α- and γ-tocopherol (μmol/mmol chol) for Male Participants Randomized to Vitamin E (N=1166) in the RAS to SELECT[a]

Variables:	Change in Plasma α-Tocopherol[b]		Change in Plasma γ-Tocopherol[c]	
	β	P-value	β	P-value
Baseline α-tocopherol (μmol/mmol free cholesterol)	-0.3639	<0.0001	-	-
Baseline γ-tocopherol (μmol/mmol free cholesterol)	-	-	-0.7371	<0.0001
Race[d]				
African American	-1.2589	<0.0001	0.2530	<0.0001
European American	Reference	-	Reference	-
Age	0.0054	0.7796	0.0001	0.9850
Smoking Dose (cigarettes/day)	-0.0141	0.6125	0.0037	0.3424
Years Smoked	0.0164	0.1577	-0.0035	0.0325
Smoking Status[e]				
Current Smokers	-0.8100	0.2708	0.1439	0.1620
Former Smokers	-0.3671	0.3073	0.0675	0.1797
Never Smokers	Reference	-	Reference	-
Body Mass Index (BMI),kg/m^2	0.0508	0.0479	0.0039	0.2883

[a] Vitamin E includes all participants on vitamin E (including vitamin E + placebo and vitamin E + selenium). RAS, Respiratory Ancillary to SELECT; SELECT, The Selenium and Vitamin E Cancer Prevention Trial. Change in plasma level defined as thirty-six month value minus the baseline value. All tocopherol values adjusted for free cholesterol at each time point. β, regression coefficient; P-value for regression coefficient.

[b] α-tocopherol outcome adjusted for baseline α-tocopherol but not γ-tocopherol. Total variability explained (R^2)=8%.

[c] γ-tocopherol outcome adjusted for baseline γ-tocopherol but not α-tocopherol. Total variability explained (R^2)=56%.

[d] European Americans serve as reference group for race.

[e] Never smokers serve as reference group for smoking status.

References

1. Otten JJ, Hellwig JP, Meyers LD. Dietary reference intakes : the essential guide to nutrient requirements. Washington, DC: National Academies Press, 2006.

2. Kelleher J, Losowsky MS. The absorption of α-tocopherol in man. British Journal of Nutrition 1970;24:1033-47.

3. Traber MG, Frei B, Beckman JS. Vitamin E revisited: do new data validate benefits for chronic disease prevention? Current opinion in lipidology. 2008;19:30-8.

4. Walsh PC. Effects of Long-Term Vitamin E Supplementation on Cardiovascular Events and Cancer: A Randomized Controlled Trial. J Urol 2005;174:1823-4.

5. The Alpha-Tocopherol Beta Carotene Cancer Prevention Study Group. The Effect of Vitamin E and Beta Carotene on the Incidence of Lung Cancer and Other Cancers in Male Smokers. N Engl J Med 1994;330:1029-35.

6. Cassano PA, Guertin KA, Kristal AR, et al. A Randomized Controlled Trial of Vitamin E and Selenium on Rate of Decline in Lung Function; in preparation.

7. Agler AH, Kurth T, Gaziano JM, Buring JE, Cassano PA. Randomised vitamin E supplementation and risk of chronic lung disease in the Women's Health Study. Thorax 2011;66:320-5.

8. Ford ES, Ajani UA, Mokdad AH. Brief Communication: The Prevalence of High Intake of Vitamin E from the Use of Supplements among U.S. Adults. Annals of Internal Medicine 2005;143:116-20.

9. Kelly FJ, Lee R, Mudawy IS. Inter- and Intra-Individual Vitamin E Uptake in Healthy Subjects Is Highly Repeatable across a Wide Supplementation Dose Range. Ann N Y Acad Sci 2004;1031:22-39.

10. Cheeseman KH, Holley AE, Kelly FJ, Wasil M, Hughes L, Burton G. Biokinetics in humans of RRR-α-tocopherol: The free phenol, acetate ester, and succinate ester forms of vitamin E. Free Radical Biology and Medicine 1995;19:591-8.

11. Roxborough HE, Burton GW, Kelly FJ. Inter- and intra-individual variation in plasma and red blood cell vitamin E after supplementation. Free Radic Res 2000;33:437-45.

12. Dimitrov NV, Meyer C, Gilliland D, Ruppenthal M, Chenoweth W, Malone W. Plasma tocopherol concentrations in response to supplemental vitamin E. The American Journal of Clinical Nutrition 1991;53:723-9.

13. Cheeseman KH, Holley AE, Kelly FJ, Wasil M, Hughes L, Burton G. Biokinetics in humans of RRR-α-tocopherol: The free phenol, acetate ester, and succinate ester forms of vitamin E. Free Radical Biology and Medicine 1995;19:591-8.

14. Dimitrov NV, Meyer C, Gilliland D, Ruppenthal M, Chenoweth W, Malone W. Plasma tocopherol concentrations in response to supplemental vitamin E. The American Journal of Clinical Nutrition 1991;53:723-9.

15. Handelman GJ, Epstein WL, Peerson J, Spiegelman D, Machlin LJ, Dratz EA. Human adipose alpha-tocopherol and gamma-tocopherol kinetics during and after 1 y of alpha-tocopherol supplementation. The American Journal of Clinical Nutrition 1994;59:1025-32.

16. Machlin LJ, Gabriel E. Kinetics of tissue alpha-tocopherol uptake and depletion following administration of high levels of vitamin E. Ann N Y Acad Sci 1982;393:48-60.

17. Burton GW, Traber MG, Acuff RV, et al. Human plasma and tissue alpha-tocopherol concentrations in response to supplementation with deuterated natural and synthetic vitamin E. The American Journal of Clinical Nutrition 1998;67:669-84.

18. Ford ES, Sowell A. Serum α-Tocopherol Status in the United States Population: Findings from The Third National Health and Nutrition Examination Survey. American Journal of Epidemiology 1999;150:290-300.

19. Ford ES, Schleicher RL, Mokdad AH, Ajani UA, Liu S. Distribution of serum concentrations of α-tocopherol and γ-tocopherol in the US population. The American Journal of Clinical Nutrition 2006;84:375-83.

20. Parker RS. Carotenoid and tocopherol composition of human adipose tissue. The American Journal of Clinical Nutrition 1988;47:33-6.

21. Hallfrisch J, Muller DC, Singh VN. Vitamin A and E intakes and plasma concentrations of retinol, (beta)-carotene, and (alpha)-tocopherol in men and women of the Baltimore Longitudinal Study of Aging. Am J Clin Nutr 1994;60:176.

22. Winkelhofer-Roob BM, Roob JM, Maritschnegg M, Sprinz G, Hiller D, et al. Does Aging Affect the Response of Vitamin E Status to Vitamin E Depletion and Supplementation? Annals of the New York Academy of Sciences 2004;1031:381-4.

23. Rock CL, Thornquist MD, Kristal AR, Patterson RE, et al. Demographic, dietary and lifestyle factors differentially explain variability in serum carotenoids and fat-soluble vitamins: Baseline results from the sentinel site of the Olestra post-marketing surveillance study. J Nutr 1999;129:855-64.

24. Davi G, Alessandrini P, Mezzetti A, et al. In Vivo Formation of 8-Epi-Prostaglandin F_{2alpha} Is Increased in Hypercholesterolemia. Arteriosclerosis, Thrombosis, and Vascular Biology 1997;17:3230-5.

, Lahmann PH, Gullberg B, Janzon L, Berglund G. Serum concentrations of beta-carotene and alpha-tocopherol are associated with diet, smoking, and general and central adiposity. Am J Clin Nutr 2001;73:777-85.

26. Bolton-Smith C, Casey CE, Gey KF, Smith WCS, Tunstall-Pedoe H. Antioxidant vitamin intakes assessed using a food-frequency questionnaire: correlation between biochemical status in smokers and non-smokers. British Journal of Nutrition 1991;65:337-46.

27. Wright ME, Lawson KA, Weinstein SJ, et al. Higher baseline serum concentrations of vitamin E are associated with lower total and cause-specific mortality in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study. Am J Clin Nutr 2006;84:1200-7.

28. Bruno RS, Ramakrishnan R, Montine TJ, Bray TM, Traber MG. Alpha-tocopherol disappearance is faster in cigarette smokers and is inversely related to their ascorbic acid status. Am J Clin Nutr 2005;81:95-103.

29. Traber MG, Winklhofer-Roob BM, Roob JM, et al. Vitamin E kinetics in smokers and nonsmokers. Free Radical Biology and Medicine 2001;31:1368-74.

30. Comstock GW, Menkes MS, Schober SE, Vuilleumier JP, Helsing KJ. Serum levels of retinol, beta-carotene, and alpha-tocopherol in older adults. Am J Epidemiol 1988;127:114-23.

31. Dietrich M, Block G, Norkus EP, et al. Smoking and exposure to environmental tobacco smoke decrease some plasma antioxidants and increase gamma-tocopherol in vivo after adjustment for dietary antioxidant intakes. Am J Clin Nutr 2003;77:160-6.

32. Stryker WS, Kaplan LA, Stein EA, Stampfer MJ, Sober A, Willet WC. The relation of diet, cigarette smoking, and alcohol consumption to plasma beta-carotene and alpha-tocopherol levels. American Journal of Epidemiology 1988;127:283-96.

33. Wei W, Kim Y, Boudreau N. Association of smoking with serum and dietary levels of antioxidants in adults: NHANES III, 1988-1994. Am J Public Health 2001;91:258-64.

, Aaran RK. Determinants of serum alpha-tocopherol in Finnish adults. Prev Med 1988;17:725-35.

-Vila P, Garcia-Closas R, Serra-Majem L, et al. Determinants of the nutritional status of vitamin E in a non-smoking Mediterranean population. Analysis of the effect of vitamin E intake, alcohol consumption and body mass index on the serum alpha-tocopherol concentration. Eur J Clin Nutr 1997;51:723-8.

36. Kimmons JE, Blanck HM, Tohill BC, Zhang J, Khan LK. Associations between body mass index and the prevalence of low micronutrient levels among US adults. Medscape general medicine 2006;8:59.

37. Myara I, Alamowitch C, Michel O, et al. Lipoprotein oxidation and plasma vitamin E in nondiabetic normotensive obese patients. Obes Res 2003;11:112-20.

38. Sinha R, Patterson BH, Mangels AR, et al. Determinants of plasma vitamin E in healthy males. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 1993;2:473-9.

39. Ascherio A, Stampfer MJ, Colditz GA, Rimm EB, Litin L, Willett WC. Correlations of Vitamin A and E Intakes with the Plasma Concentrations of Carotenoids and Tocopherols among American Men and Women. The Journal of Nutrition 1992;122:1792-801.

40. Keaney JF Jr, Larson MG, Vasan RS, et al. Obesity and systemic oxidative stress: clinical correlates of oxidative stress in the Framingham Study. Arterioscler Thromb Vasc Biol 2003;23:434-9.

41. Borel P, Moussa M, Reboul E, et al. Human Plasma Levels of Vitamin E and Carotenoids Are Associated with Genetic Polymorphisms in Genes Involved in Lipid Metabolism. The Journal of Nutrition 2007;137:2653-9.

42. Reboul E, Klein A, Bietrix F, et al. Scavenger receptor class B type I (SR-BI) is involved in vitamin E transport across the enterocyte. The Journal of biological chemistry 2006;281:4739-45.

43. Major JM, Yu K, Wheeler W, et al. Genome-wide association study identifies common variants associated with circulating vitamin E levels. Hum Mol Genet 2011;20:3876-83.

44. Wright ME, Peters U, Gunter MJ, et al. Association of Variants in Two Vitamin E Transport Genes with Circulating Vitamin E Concentrations and Prostate Cancer Risk. Cancer Research 2009;69:1429-38.

45. Zingg J, Azzi A, Meydani M. Genetic polymorphisms as determinants for disease-preventive effects of vitamin E Nutr Rev 2008;66:406 <last_page> 414.

46. Major J.M., Yu K., Weinstein S.J., et al. Genome-wide association study identifies three common variants associated with serologic response to Vitamin E supplementation in men. Journal of Nutrition 2012;142:866-71.

47. Leonard PJ, Shaper AG, Jones KW. Relationship between free and total cholesterol values in human serum. Am J Clin Nutr 1965;17:377-80.

48. Lopez A, Krehl WA, Hodges RE. Relationship between total cholesterol and cholesteryl esters with age in human blood plasma. Am J Clin Nutr 1967;20:808-15.

49. Agler AH, Crystal RG, Mezey JG, et al. Differential Expression of Vitamin E and Selenium-Responsive Genes by Disease Severity in Chronic Obstructive Pulmonary Disease. COPD: Journal of Chronic Obstructive Pulmonary Disease 2013;10:1-9.

50. Morrow JD, Frei B, Longmire AW, et al. Increase in Circulating Products of Lipid Peroxidation (F2-Isoprostanes) in Smokers — Smoking as a Cause of Oxidative Damage. N Engl J Med 1995;332:1198-203.

51. Helmersson J, Ärnlöv J, Vessby B, Larsson A, Alfthan G, Basu S. Serum selenium predicts levels of F2-isoprostanes and prostaglandin F2α in a 27 year follow-up study of Swedish men. Free Radical Research 2005;39:763-70.

52. Guertin K, Kristal A, Grant R, et al.
Effect of Vitamin E and Selenium on Urine F_2-Isoprostanes,A Biomarker of Oxidative Stress; in preparation.

53. Sharma G, Muller DP, O'Riordan SM, et al. Urinary conjugated α-tocopheronolactone—a biomarker of oxidative stress in children with type 1 diabetes. FRB Free Radical Biology and Medicine 2012;

54. Faruque O, Khan MR, Rahman M, Ahmed F. Relationship between smoking and antioxidant nutrient status. Br J Nutr 1995;73:625.

55. Proteggente AR, Rota C, Majewicz J, et al. Cigarette smokers differ in their handling of natural (RRR) and synthetic (all rac) α-tocopherol: A biokinetic study in apoE4 male subjects. Free Radical Biology and Medicine 2006;40:2080-91.

56. Park Y. The metabolic syndrome: prevalence and associated risk factor findings in the US population from the Third National Health and Nutrition Examination Survey, 1988-1994. Archives of internal medicine (1960) 2003;163:427-36.

57. Green MS, Heiss G, Rifkind BM, Cooper GR, Williams OD, Tyroler HA. The ratio of plasma high-density lipoprotein cholesterol to total and low-density lipoprotein cholesterol: age-related changes and race and sex differences in selected North American populations. The Lipid Research Clinics Program Prevalence Study. Circulation 1985;72:93-104.

58. Doring F, Rimbach G, Lodge JK. In silico search for single nucleotide polymorphisms in genes important in vitamin E homeostasis IUBMB Life 2004;56:615-20.

59. Bardowell SA, Stec DE, Parker RS. Common variants of cytochrome P450 4F2 exhibit altered vitamin E-{omega}-hydroxylase specific activity. J Nutr 2010;140:1901-6.

60. Stec DE, Roman RJ, Flasch A, Rieder MJ. Functional polymorphism in human CYP4F2 decreases 20-HETE production. Physiological genomics 2007;30:74-81.

61. Handelman GJ, Machlin LJ, Fitch K, Weiter JJ, Dratz EA. Oral α-Tocopherol Supplements Decrease Plasma γ-Tocopherol Levels in Humans Journal of Nutrition 1985;115:807-13.

62. Traber MG, Rader D, Acuff RV, Ramakrishnan R, Brewer HB, Kayden HJ. Vitamin E dose-response studies in humans with use of deuterated RRR-alpha-tocopherol. The American Journal of Clinical Nutrition 1998;68:847-53.

63. Schultz M, Leist M, Petrzika M, Gassmann B, Brigelius-Flohé R. Novel urinary metabolite of alpha-tocopherol, 2,5,7,8-tetramethyl-2(2'-carboxyethyl)-6-hydroxychroman, as an indicator of an adequate vitamin E supply? The American Journal of Clinical Nutrition 1995;62:1527S-34S.

64. The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group. The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. N Engl J Med 1994;330:1029-35.

65. Wagner K, Kamal-Eldin A, Elmadfa I. Gamma-Tocopherol-- An Underestimated Vitamin? Annals of Nutrition & Metabolism 2004;48:169-88.

ONLINE SUPPLEMENTAL MATERIALS

Supplemental Methods:

Supplemental Table 1 Baseline Characteristics of Male Participants Randomized to Placebo (n=89) in the RASto SELECT[a]

	Placebo (n=89)
Age (y)	63.3 ± 6.8[b]
Ethnicity [n (%)]	
European American	70 (79)
African American	19 (21)
Smoking Status[c] [n(%)]	
Never	32 (36)
Former	46 (52)
Current	10 (11)
Body Mass Index (BMI), kg/m^2	29.0 ± 5.3
BMI[d] [n(%)]	
Normal	21 (24)
Overweight	37 (41)
Obese	31 (35)
Free cholesterol (mmol/L)[e]	2454.4 ± 1239.9 2171.7(1025.9, 9520.3)[f]

[a] Placebo refers to participants taking both placebo pills (vitamin E placebo + selenium placebo). RAS, Respiratory Ancillary to SELECT. SELECT, The Selenium and Vitamin E Cancer Prevention Trial. 89 of 726 men on placebo had vitamin E assayed.

[b] Mean ± SDs.

[c] 6 participants missing baseline smoking data (n=1 double placebo, n=1 Vitamin E plus Selenium, n=4 Vitamin E plus placebo).

[d] Obese defined as BMI ≥30. Overweight defined as BMI 25-30. Normal weight (BMI <25) serves as reference group. This sample did not include underweight individuals.

[e] Free cholesterol refers to unesterified lipids.

[f] Median; minimum, maximum in parentheses.

Supplemental Table 2 Plasma Tocopherol Concentration at Thirty-six Months on Supplement in Men Randomized to Placebo (N=89) in the RAS to SELECT[a]

		Plasma Tocopherol at Study Baseline	Plasma Tocopherol at 36 months	Change in Plasma Tocopherol[b]
α-tocopherol	Unadjusted, μmol/L	22.5 ± 11.2[c]	19.5 ± 8.3	-3.0 ± 7.9
		20.7 (8.7, 80.8)[d]	16.9 (7.1, 47.5)	-2.2 (-44.2, 15.7)
	Adjusted, μmol/mmol chol	9.5 ± 2.9	8.7 ± 2.3	-0.8 ± 2.1
		9.1 (5.0, 20.9)	8.5 (3.6, 15.9)	-0.5 (-7.7, 3.4)
γ-tocopherol	Unadjusted, μmol/L	3.5 ± 2.8	3.9 ± 3.5	0.4 ± 0.8
		3.0 (0.2, 20.1)	3.1 (0.4, 25.7)	0.1 (-6.9, 19.6)
	Adjusted, μmol/mmol chol	1.5 ± 0.9	1.6 ± 0.8	0.1 ± 0.8
		1.5 (0.1, 5.9)	1.6 (0.2, 4.7)	0.2 (-2.4, 2.0)
α-tocopherol/ γ-tocopherol ratio		11.3 ± 16.5	7.7 ± 7.5	-3.6 ± 15.3
		7.3 (1.4, 130.1)	5.5 (1.8, 47.7)	-0.89 (-122.9, 30.4)

[a] Placebo refers to participants taking both placebo pills (vitamin E placebo + selenium placebo). RAS, Respiratory Ancillary to SELECT. SELECT, The Selenium and Vitamin E Cancer Prevention Trial.

[b] Change in plasma level defined as thirty-six month value minus the baseline value. RAS, Respiratory Ancillary to SELECT. SELECT, The Selenium and Vitamin E Cancer Prevention Trial. 89 of 726 men on placebo had vitamin E assayed.

[c] Mean ± SDs.

[d] Median; minimum, maximum in parentheses.

Supplemental Table 3 Baseline Plasma Tocopherol Levels in Men Randomized to Vitamin E in the RAS to SELECT[a]

	α-tocopherol (μmol/mmol chol)	γ-tocopherol (μmol/mmol chol)
Ethnicity [*n* (%)]		
European American	9.2 ± 3.0 8.6 (4.1, 29.8)	1.4 ± 0.8 1.2 (0.1, 6.2)
African American	8.1 ± 2.9 7.4 (3.6, 22.3)	1.9 ± 0.9 1.8 (0.1, 5.2)
Smoking Status [*n* (%)][b]		
Never	9.1 ± 3.0 1.3 (0.1, 6.2)	1.5 ± 0.9 1.3 (0.1, 6.2)
Former	9.1 ± 3.1 8.4 (4.4, 29.8)	1.5 ± 0.9 1.3 (0.1, 5.2)
Current	8.0 ± 2.8 7.3 (4.0, 18.6)	1.6 ± 0.9 1.4 (0.1, 4.4)
Body Mass Index (BMI), kg/m^2[c]		
Normal	8.9 ± 3.0 8.2 (3.6, 21.2)	1.3 ± 0.9 1.2 (0.1, 4.5)
Overweight	8.9 ± 2.9 8.3 (4.3, 25.8)	1.4 ± 0.9 1.3 (0.1, 5.2)
Obese	9.0 ± 3.3 8.2 (4.0, 29.8)	1.6 ± 0.9 1.5 (0.1, 6.2)

[a] Vitamin E includes all participants on vitamin E (including vitamin E + placebo and vitamin E + selenium, n=1166). RAS, Respiratory Ancillary to SELECT. SELECT, The Selenium and Vitamin E Cancer Prevention Trial. Values shown are mean ± SDs on first line, and median (minimum, maximum)

[b] Never smokers n=378, former smokers n=585, current smokers n=185.

[c] BMI (kg/m^2). Obese defined as BMI ≥30, overweight defined as BMI 25-30, normal weight (BMI <25) serves as reference group. This sample did not include underweight individuals.

Supplemental Table 4 Model-based Predicted Change in Plasma Tocopherols (μmol α/mmol chol) Among Men Randomized to Vitamin E (400 IU/day *all rac*-α-tocopherol) in the RAS to SELECT[a]

Variables in Model	Predicted Change in Plasma α-Tocopherol (μmol/mmol chol)		Predicted Change in Plasma γ-Tocopherol (μmol/mmol chol)	
	European American	African American	European American	African American
Smoking Status				
Never	4.1 ± 1.1[b] 4.3 (-0.2, 5.8)	3.2 ± 1.2 3.4 (-1.5, 4.9)	-0.6 ± 0.6 -0.5 (-4.1, 0.3)	-0.8 ± 0.6 -0.7 (-2.6, 0.5)
Former	4.1 ± 1.1 4.2 (-3.4, 5.8)	3.0 ± 1.1 3.4 (-1.9, 4.6)	-0.6 ± 0.6 -0.5 (-3.9, 0.3)	-0.8 ± 0.7 -0.7 (-3.2, 0.3)
Current	3.8 ± 1.1 4.0 (0.2, 5.5)	3.1 ± 0.8 3.3 (0.9, 4.3)	-0.6 ± 0.6 -0.4 (-2.4, 0.3)	-0.7 ± 0.7 -0.7 (-2.6, 0.5)

[1] Vitamin E includes all participants on vitamin E (including vitamin E + placebo and vitamin E + selenium, n=1166). RAS, Respiratory Ancillary to SELECT. SELECT, The Selenium and Vitamin E Cancer Prevention Trial. Change in plasma level defined as thirty-six month value minus the baseline value. All tocopherol values adjusted for free cholesterol at each time point. Unless otherwise noted, models predicting change in α-tocopherol adjust for baseline α-tocopherol level, and models predicting change in γ-tocopherol adjust for baseline γ-tocopherol level. Models adjusted for all other variables, including smoking status, smoking dose, years smoked, age, race, and BMI. [b]Values shown are mean ± SDs on first line, and median (25th percentile, 75th percentile)

Supplemental Table 5 Regression Coefficients from Mixed Models (Unadjusted Models) for Change in Plasma α- and γ-tocopherol (μmol/mmol chol) for Male Participants Randomized to Vitamin E in the RAS to SELECT[a]

Variables	Change in Plasma α-Tocopherol[b]		Change in Plasma γ-Tocopherol[c]	
	β	P-value	β	P-value
Baseline α-tocopherol	-0.3308	(<0.0001)	0.0147	(0.0094)
Baseline γ-tocopherol	1.0645	(<0.0001)	-0.6868	(<0.0001)
Race[d]				
African American	-1.33	(<0.0001)	0.2569	(<0.0001)
European American	Reference	-	-	-
Age	0.0268	(0.1516)	-0.0048	(0.0688)
Smoking Status[e]	Type III p-value=0.1000			
Current Smokers	-0.7426	(0.0439)	0.1295	(0.0122)
Former Smokers	-0.0634	(0.8130)	0.0083	(0.8252)
Never Smokers	Reference	-	-	-
Smoking Dose	-0.0269	(0.0776)	0.0049	(0.0220)
Years Smoked	-0.0037	(0.6066)	0.0004	(0.6862)
Body Mass Index (BMI), kg/m^2	0.0526	(<0.0001)	0.0029	(0.4216)
BMI Category[f]	Type III p-value=0.3776		Type III p-value=0.1883	
Obese	0.4624	(0.1771)	0.0481	(0.3221)
Overweight	0.2136	(0.5123)	-0.0211	(0.6450)
Normal weight	Reference	-	-	-

[a] Vitamin E includes all participants on vitamin E (including vitamin E + placebo and vitamin E + selenium, n=1166). RAS, Respiratory Ancillary to SELECT; SELECT, The Selenium and Vitamin E Cancer Prevention Trial. Change in plasma level defined as thirty-six month value minus the baseline value. All tocopherol values adjusted for free cholesterol at each time point. Models adjust for baseline tocopherol level unless otherwise noted. β, regression coefficient for each variable adjusted only for baseline tocopherol (unadjusted for other variables); P-value for regression coefficient.

[b] α-tocopherol outcome adjusted for baseline α-tocopherol but not γ-tocopherol.

[c] γ-tocopherol outcome adjusted for baseline γ-tocopherol but not α-tocopherol.

[d] European Americans serve as reference group for race.

[e] Never smokers serve as reference group for smoking status.

[f] Obese defined as BMI ≥30. Overweight defined as BMI 25-30. Normal weight (BMI <25) serves as reference group. This sample did not include underweight individuals.

CHAPTER 3

THE CONTRIBUTION OF BASELINE NUTRITURE TO INTERVENTION EFFECTS IN A RANOMIZED CONTROLLED TRIAL

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Running Head: Effects of Vitamin E and Selenium on Lung Function Decline

Keywords: selenium, antioxidant nutrients, randomized controlled trial, vitamins/administration & dosage, lung function

Abstract

Background Randomized controlled trials are the gold-standard for tests of hypothesis, however in order to fully assess the effect of an intervention intent-to-treat analyses are complemented by as-treated and plausibility analyses.

Methods Using data from the Respiratory Ancillary Study (RAS) to the Selenium and Vitamin E Cancer Prevention Trial (SELECT), we investigated the effects of baseline selenium nutriture on the effect of selenium supplementation (200 μg/day L-selenomethionine) on rate of decline in lung function. Plasma selenium was assessed in 2,681 men at study baseline, prior to supplementation, and rate of decline in lung function was assessed by longitudinal spirometry measures over approximately three years.

Results Treatment with selenium had no effect on rate of decline in the forced expiratory volume in the first second (FEV_1), and considering baseline nutriture there was no effect even in men with lower baseline selenium. Furthermore, in the untreated placebo arm there was no association of baseline selenium with rate of decline in lung function.

Conclusions Selenium does not attenuate rate of decline in lung function, with or without consideration of baseline nutriture. The higher overall baseline distribution of selenium in this study (mean 170 ng/mL, SD 28) precludes a complete assessment of selenium effects in a sample with low selenium.

Introduction

Chronic Obstructive Pulmonary Disease (COPD) is an important cause of morbidity and mortality in the United States(1), and its prevalence is increasing(2). An imbalance in oxidant-antioxidant levels is proposed to play a key role in the pathogenesis of COPD, and individuals with COPD have higher levels of oxidative stress. Nutrients with antioxidant properties, including selenium(3), are hypothesized to play a role in preventing and/or attenuating oxidative stress-related diseases, including COPD. Some nutrients, in particular selenium, are only modifiable through supplementation because of variation in food sources and/or lack of specific information on nutrient content of foods, thus supplementation remains a viable method to mitigate oxidative damage and reduce disease risk. Currently available therapies for COPD show only modest benefits in ameliorating symptoms, and most treatments do not slow the rate of decline in lung function that is the hallmark of this disease(4-6), highlighting the important need for novel preventive and therapeutic approaches, including nutritional modification.

The key symptom of COPD is rapid decline in lung function, and lung function, measured by spirometry, is the cornerstone of COPD diagnosis and severity staging. The Respiratory Ancillary Study (RAS) to the Selenium and Vitamin E Cancer Prevention Trial (SELECT) investigated the effect of intervention with vitamin E and/or selenium on rate of decline in lung function and reported no effect of selenium on rate of decline(7). This randomized trial was null in spite of strong cross-sectional evidence that higher plasma selenium was associated with better lung function in NHANES III(8). Prior clinical trials of selenium identified a selenium nutriture threshold, below which effects of selenium supplementation on cancer risk were evident(9). The question of whether response to

intervention is modified by baseline level of the nutrient comprising the intervention is a key question, with important implications for all trials of nutrient supplementation(10, 11), and is a key consideration in the context of interpretation of null results from randomized trials.

Selenium has antioxidant functions and is a constituent in protective enzymes and amino acids, including selenocysteine and selenomethionine(12). Higher selenium status was associated with lower levels of oxidative stress biomarkers in observational studies(13), but findings from selenium intervention studies were mixed(14-16). Only a handful of past studies assess the association of selenium with lung outcomes, but there is evidence for protective associations for lung function(8) and asthma risk(17-19).

Considering that prior studies of selenium int(19)ervention found effects were limited to individuals with low baseline selenium status(20), we investigated similar hypotheses in the RAS. Thus, we sought to complete a more comprehensive investigation of the effect of supplements, beyond the intent-to-treat model in the main analysis of the trial(7). Using baseline nutriture, measured as plasma selenium concentration in pre-randomization blood samples, we investigated whether baseline selenium nutriture modified the effect of selenium supplementation on rate of decline in lung function.

Methods

Study Population

The Respiratory Ancillary Study (RAS) to the Selenium and Vitamin E Cancer Prevention Trial (SELECT) investigated the effects of vitamin E and selenium on the annual rate of decline in pulmonary function. SELECT was a prostate cancer primary prevention

trial which randomized 35,433 male participants ages fifty and older at 428 study sites in North America to four arms: 400 IU/day α-tocopherol (*all rac*-α-tocopheryl acetate, hereafter referred to as vitamin E) plus selenium placebo; vitamin E placebo plus 200 μg/day selenomethionine (L-selenomethionine, selenium); both active supplements; or, vitamin E placebo plus selenium placebo(21). Cigarette smokers were preferentially recruited to the RAS given their higher risk for steeper lung function decline(7).

Using a post-randomization design, the RAS studied multiple pulmonary function parameters: forced expiratory volume in the first second (FEV_1), forced expiratory flow at 25 to 75% of the forced vital capacity (FVC) (FEF_{25-75}); and the ratio of FEV_1/FVC. The informative pulmonary function parameters are indicators of irreversible airflow limitation, which occurs in individuals with COPD.

Measurements

Spirometry was completed following The American Thoracic Society (ATS) guidelines(22), using a handheld electronic spirometer (EasyOne, ndd Medizintechnik, MA). Pulmonary function was assessed about once per year, at least three times over four years, however early withdrawal of supplement on October 23, 2008 resulted in fewer assessments during the supplemented period; pulmonary function tests prior to March 1, 2009 were considered to reflect treatment effects on the rate of decline. Pulmonary function testing was under strict quality control, and details are reported elsewhere(7).

*S*ample preparation and analysis for selenium was performed at the USDA Plant, Soil and Nutritional Research Unit, Cornell University, Ithaca, NY. A 2% nitric acid solution was prepared using concentrated nitric acid from a Teflon sub-boiling still and double-deionized water, resistivity > 18 MΩ cm. Plasma samples were prepared for selenium

analysis by adding 5 mL of 2% HNO_3 to 100 µL of plasma (thawed on ice). Assessment of accuracy and precision were determined by assaying a 10 ng/mL reference standard and a mixed plasma control sample periodically throughout all sample runs. The mixed plasma control samples were prepared in the same way as the plasma samples. An Agilent 7500 cs/ce quadrupole inductively coupled plasma-dynamic reaction cell-mass spectrometry (ICP-MS) equipped with a dynamic reaction cell was used to determine selenium concentration in plasma samples. The ion intensity at *m/z* 78 was used to monitor Se and Germanium at m/z 72 was used as an internal standard. Data were collected using the *Spectrum* mode for direct analysis. Maximum sensitivity was obtained by daily tuning of the ion lens system, gas flow rates, and other parameters. Samples were run manually with continuous quality monitoring.

Statistical Methods

All analyses were carried out in SAS, version 9.3 (SAS Institute, Cary, NC). Age, height, time (elapsed years) and lung function were continuous variables, and smoking status, race/ethnicity, and treatment arm were categorical variables. Baseline selenium was considered as a continuous variable; in addition, we also categorized selenium into quartiles and tertiles, assessing both linear trends and non-linear associations.

Hierarchical linear regression models (mixed models) estimated the effect of intervention on repeated measurements of lung function, and mediation by baseline nutriture was assessed by adding an interaction term between baseline plasma selenium, treatment, and time. Refer to Appendix for further details on model selection and checking.

Results

Plasma selenium was assayed in blood samples collected at SELECT baseline (pre-randomization) for 2,681 participants in the RAS; among these men, 38% reported use of a supplement containing selenium in the year prior to randomization. The mean age was 63 (SD 6) years, 22% were African American, and 16% of participants were current smokers; participant characteristics did not vary by treatment arm (Table 1).

At the baseline, the mean plasma selenium concentration was 170 (SD 28) ng/mL, and there were no differences by treatment arm (Table 1). Using a definition of low selenium, which was developed in the Nutritional Prevention of Cancer Study (Se < 123.2 ng/mL= low selenium)(9), only 1.6% of the RAS participants had low selenium (data not shown). Considering the distribution of baseline plasma selenium concentration across all RAS participants with measurements, the mean of each quintile (minimum, maximum) was: quintile I, 138 (90, 149); quintile II, 156 (149, 163); quintile III, 168 (163, 174); quintile IV, 181 (174, 189); and quintile V, 210 (189, 459) ng/mL.

Effect of baseline nutriture on treatment effect

The effect of treatment on rate of decline in lung function did not differ by baseline plasma selenium concentration (Table 2). A comprehensive analytic approach considered the effect of any selenium (selenium alone or selenium + vitamin E) compared to placebo, the effect of selenium alone compared to placebo, and the effect of selenium + vitamin E compared to placebo. Findings were the same whether selenium was considered as a continuous variable (ng/mL) or as a categorical variable, coded as ordered categories or as a nominal variable. Categorical models considered low selenium versus selenium replete groups, and either tertiles of quintiles of the distribution. The low selenium models were

under-powered given only 1.6% of men had low selenium using definitions identified in prior studies of chronic disease outcomes.

Given that treatment with vitamin E attenuated the rate in decline in FEV_1, models considering effect modification by baseline selenium nutrition in the test of selenium + vitamin E placebo versus placebo + placebo are preferred because they are unaffected by vitamin E supplementation. Findings for all models considered (Table 2) are consistent; there is no evidence for effect modification by baseline selenium nutriture (p-values for model tests of effect modification are shown in Table 2), and, furthermore, the model coefficients do not reveal any evidence for effect modification that is of an interesting magnitude and/or in the hypothesized direction.

Effect of baseline plasma selenium on cross-sectional lung function and rate of decline

Men in the placebo only arm (n=658) were studied to investigate the association of baseline plasma selenium concentration with lung function, independent of any effects of treatment, and considering both the cross-sectional and longitudinal outcomes. Selenium was treated as both as a continuous and categorical variable in these analyses. There was no evidence that starting plasma concentration of selenium was associated with subsequent rate of decline in FEV_1 in men in the double placebo arm (Table 3).

Discussion

There was no evidence for effect modification by usual selenium nutrition; thus, the effect of selenium intervention on rate of decline in FEV_1, which was null overall, did not differ by usual selenium nutriture. Neither was there evidence that usual selenium nutriture was associated with the subsequent rate of decline in FEV_1 in the untreated placebo group. This paper resolves the possibility of differential potential to benefit in the

interpretation of the null result for selenium in the RAS(7). While past findings from observational studies supported a positive association of plasma selenium with lung function(8), the randomized placebo-controlled design, as used herein, allows for a stronger test of the hypothesis for an effect of selenium, and both the intent-to-treat and the potential to benefit analysis are clearly null.

NHANES III findings supported a clear and strongly positive cross-sectional association of serum selenium with lung function (8), but in RAS analyses limited to the placebo arm there was no cross-sectional association of plasma selenium concentration with lung function. Furthermore, selenium alone did not lower *in vivo* oxidative stress, as indicated by effects on a urine biomarker (F_2-isoprostanes) in a previous study of participants in the RAS(16).

A recent workshop on evidence-based nutrition emphasized that need to carefully consider the randomized controlled trial design for investigation of nutrient-disease questions because of the inability to identify and use a truly unexposed (no intake) control group(10). SELECT, however, supplemented with pharmacological doses of selenium(21), which led to plasma concentrations above those obtained through dietary manipulation alone. Furthermore, a specially formulated multivitamin was provided to SELECT men and their spouses to ensure that self-supplementation with multivitamins, which is common in the United States, did not include the study nutrients. Thus, nutrient intake in the active treatment groups was expected to derive mainly from the study supplement, and intake was therefore far above the dietary intake in the placebo group, allowing for a strong test of hypothesis.

A second point in using the randomized trial design to study nutrient intervention is the concept of potential to benefit(10). The evidence base that identifies the optimal level of nutrients for the prevention of chronic disease is weak, and thus in identifying and registering trial participants there is no screening for nutritional status, nor any consideration of usual nutriture for the supplemented nutrients. In the case of the RAS and SELECT, men were right-shifted in their distribution of selenium nutriture; median levels were 168 and 135 ng/mL(21), respectively. Only 1.6% of RAS men were below thresholds established in a prior study as representing thresholds below which intervention would be expected to have positive benefits(9). Similarly, in comparing the distribution of selenium in the RAS participants to the distribution of selenium in NHANES III adult participants, there is far less variability in the RAS and this may contribute to the null findings; the average selenium reported in NHANES III was 123 ng/mL (SD 17) (8), confirming the right-shift in the RAS distribution of plasma selenium at baseline (mean 170 ng/mL (SD 28)). Of note, selenium values in NHANES(8) III used the atomic absorption spectrophotometry method, however a more recent analysis among adults ≥40 years old in NHANES 2003-2004 reported a mean selenium of 136.7 ng/mL and also used ICP-MS(23). The high levels of baseline plasma selenium in RAS are not, therefore, an artifact of the laboratory assay, as RAS and NHANES 2003-2004 both utilized ICP-MS.

The data used herein possess many of the qualities, put forth by Blumberg et al(10), which enhance certainty of randomized controlled trial results; these qualities include minimal loss of sampling units, due to high retention in SELECT and RAS. These data also allow for adequate contrast in intake between the intervention and control group, since SELECT provided participants with a free multivitamin, free of the nutrients under study,

for the man and their spouse(21), thus ensuring that selenium intake at or near supplemented ranges for selenium was limited to the active treatment arms.

The data utilized herein to investigate the association between baseline selenium and lung function, including and excluding the effect of treatment, is valuable given longitudinal lung function measurements, long-term supplementation, and inclusion of African Americans and smokers. The null findings are similar to and extend the previously reported findings for the full RAS study(7); thus, we can now conclude that selenium does not attenuate rate of decline in lung function with or without consideration of baseline selenium nutriture. This study cannot rule out the possibility that persons with low baseline selenium, as defined in past studies(9), benefit from supplementation with selenium; too few participants in RAS were below this level to allow a test of this hypothesis.

Table 1 Baseline Characteristics of Study Participants in the RAS to SELECT[a]

Characteristics	Placebo (n=658)	Selenium (n=702)
Age at SELECT randomization, years [n(%)]	63 (7)	63 (6)
Race/ethnicity [n(%)]		
African American	134 (20)	165 (24)
European American/Other	524 (80)	537 (76)
Smoking status[n(%)][b]		
Never/Former	550 (84)	600 (85)
Current	103 (16)	97 (14)
Selenium Supplement Use [n(%)]		
0 (none)	394 (60)	427 (62)
≥50 IU	57 (9)	53 (8)
≥ 200 IU	9 (1)	8 (1)
Low Plasma Selenium Pre-randomization (<11.6 ng/mL) [n(%)]	8 (1)	17 (2)
Plasma Selenium Pre-randomization (ng/mL) Quintile:[c]		
1	139 (9)	137 (12)
2	156 (4)	157 (4)
3	169 (3)	168 (3)
4	181 (4)	181 (4)
5	210 (35)	209 (23)
Plasma Selenium Pre-randomization (ng/mL) Tertile:[c]		
1	144 (10)	143 (13)
2	168 (6)	168 (5)
3	199 (30)	198 (22)

[a]RAS, Respiratory Ancillary Study; SELECT, Selenium and Vitamin E Cancer Prevention Trial; 2,681 men with selenium assayed at SELECT baseline by ICP-MS method (n=658 placebo, 702 selenium; 667 vitamin E and 654 vitamin E plus selenium, not shown). Mean (standard deviation) unless otherwise noted

[b]Participants missing smoking: n=5 selenium, n=5 placebo arm

[c]Quintiles and tertiles cutpoints from the full distribution of participants with selenium assayed (n=2,681 across all four treatment arms)

Table 2. Effect Modification by Baseline Selenium of Selenium Treatment Effect on Rate of Decline in FEV_1 in the RAS to SELECT: Selenium only versus Placebo only[a]

Baseline Plasma Selenium	P-Value[b]
Continuous variable	0.7050
Quintile variable[c]	
Continuous	0.4138
Categorical[d]	0.5962
Tertile variable[e]	
Continuous	0.7777
Categorical[d]	0.8372

[a]RAS, the Respiratory Ancillary Study; SELECT, Selenium and Vitamin E Cancer Prevention Trial; models shown are Se only (no vitamin E) compared to placebo (neither Se or vitamin E)

[b]P-value shown for interaction terms from hierarchical linear mixed models; 3-way interaction of time * treatment * baseline selenium

[c]Quintiles of baseline selenium defined by cutpoints: 149, 162, 173, 188 ng/mL.

[d]Type III p-value

[e]Tertiles of selenium defined by cutpoints 158, 178 ng/mL.

References

1. Institute NHLaB. Morbidity and mortality: Chart book on cardiovascular, lung and blood diseases. 2009;November 8 2012. Internet: http://www.nhlbi.nih.gov/resources/docs/2009_ChartBook_508.pdf

2. Lopez AD, Shibuya K, Rao C, et al. Chronic obstructive pulmonary disease: current burden and future projections Eur Respir J 2006;27:397-412.

3. Davis CD, Tsuji PA, Milner JA. Selenoproteins and cancer prevention. Annu Rev Nutr 2012;32:73-95.

4. Anthonisen NR, Connett JE, Murray RP. Smoking and Lung Function of Lung Health Study Participants after 11 Years. Am J Respir Crit Care Med 2002;166:675-9.

5. Croxton TL, Weinmann GG, Senior RM, Wise RA, Crapo JD, Buist AS. Clinical Research in Chronic Obstructive Pulmonary Disease: Needs and Opportunities. Am J Respir Crit Care Med 2003;167:1142-9.

6. Barnes PJ. Future Treatments. In: Currie, GP, ed. ABC of COPD. UK: John Wiley & Sons, 2011:72-75.

7. Cassano PA, Guertin KA, Kristal AR, et al. A Randomized Controlled Trial of Vitamin E and Selenium on Rate of Decline in Lung Function; in preparation.

8. Hu G, Cassano PA. Antioxidant Nutrients and Pulmonary Function: The Third National Health and Nutrition Examination Survey (NHANES III). American Journal of Epidemiology 2000;151:975-81.

9. Duffield-Lillico AJ, Dalkin BL, Reid ME, et al. Selenium supplementation, baseline plasma selenium status and incidence of prostate cancer: an analysis of the complete treatment period of the Nutritional Prevention of Cancer Trial. BJU Int 2003;91:608-12.

10. Blumberg J., Heaney R.P., Huncharek M., et al. Evidence-based criteria in the nutritional context. Nutr.Rev.Nutrition Reviews 2010;68:478-84.

11. Habicht JP, Victora CG, Vaughan JP. Evaluation designs for adequacy, plausibility and probability of public health programme performance and impact. Int J Epidemiol 1999;28:10-8.

12. Brenneisen P, Steinbrenner H, Sies H. Selenium, oxidative stress, and health aspects. Mol Aspects Med 2005;26:256-67.

13. Helmersson J, Ärnlöv J, Vessby B, Larsson A, Alfthan G, Basu S. Serum selenium predicts levels of F2-isoprostanes and prostaglandin F2α in a 27 year follow-up study of Swedish men. Free Radical Research 2005;39:763-70.

14. Mishra V, Baines M, Perry SE, et al. Effect of selenium supplementation on biochemical markers and outcome in critically ill patients. Clinical Nutrition 2007;26:41-50.

15. Wu J, Salisbury C, Graham R, Lyons G, Fenech M. Increased consumption of wheat biofortified with selenium does not modify biomarkers of cancer risk, oxidative stress, or immune function in healthy Australian males. Environ Mol Mutagen 2009;50:489-501.

16. Guertin K, Kristal A, Grant R, et al. Effect of Vitamin E and Selenium on Urine F_2-Isoprostanes,A Biomarker of Oxidative Stress; in preparation.

17. Burney P, Potts J, Makowska J, et al. Original article: A case-control study of the relation between plasma selenium and asthma in European populations: a GA^{2}LEN project. Allergy 2008;63:865-71.

18. Shaheen SO, Sterne JA, Thompson RL, Songhurst CE, Margetts BM, Burney PG. Dietary antioxidants and asthma in adults: population-based case-control study. American journal of respiratory and critical care medicine 2001;164:1823-8.

19. Shaheen SO, Newson RB, Rayman MP, et al. Randomised, double blind, placebo-controlled trial of selenium supplementation in adult asthma. Thorax 2007;62:483-90.

20. Rayman MP. Selenium and human health. The Lancet 379:1256-68.

21. Lippman SM, Goodman PJ, Klein EA, et al. Designing the Selenium and Vitamin E Cancer Prevention Trial (SELECT) J Natl Cancer Inst 2005;97:94-102.

22. American Thoracic Society. Standardisation of spirometry. European Respiratory Journal 2005;26:319-38.

23. Laclaustra M, Stranges S, Navas-Acien A, Ordovas JM, Guallar E. Serum selenium and serum lipids in US adults: National Health and Nutrition Examination Survey (NHANES) 2003-2004. Atherosclerosis 2010;210:643-8.

CHAPTER 5

CONCLUSION

A randomized controlled trial is considered to be the gold-standard for the test of a hypothesis, however analyses beyond intent-to-treat are needed to fully consider the plausibility of intervention effects, particularly in the context of null findings(1). Sample characteristics and mechanisms of action are two important considerations for the interpretation of intervention effects. The analyses described herein utilized data from the Respiratory Ancillary Study (RAS) to the Selenium and Vitamin E Cancer Prevention Trial, a long-term randomized placebo-controlled trial of men assigned to vitamin E (400 IU/day *all rac*-α-tocopheryl acetate) and/or selenium (200 μg/day L-selenomethionine). While RAS found no effect of selenium on rate of decline in lung function, a non-statistically significant main effect of vitamin E to attenuate rate of decline in lung function, and a statistically significant effect of vitamin E in cigarette smokers (2), this dissertation further investigated the plausibility of effects of these nutrients in a three-tiered approach. First, effects of intervention on a biomarker of oxidative stress were assessed; secondly, we considered associations between participant characteristics and change in plasma vitamin E levels; and, finally, we considered whether there was an effect of selenium on rate of decline in lung function in men with low baseline selenium nutriture.

The RAS data allowed for a strong test of these hypotheses given the randomized trial design, long-term supplementation, and longitudinal pulmonary function measurements, and thus the investigations presented in this dissertation address gaps in the literature by improving upon by considering effects of these nutrients more completely. Sample characteristics are also a strength of this study; African Americans, traditionally

under-represented in epidemiological studies, were intensively recruited by SELECT and comprised 24% of RAS participants(2). Furthermore, purposeful oversampling of cigarette smokers by RAS (16% current smokers, 48% former smokers)(2) allowed for detection of effects of treatment on an oxidative stress biomarker, given that smokers have higher levels of oxidative stress and thus had greater potential to benefit from intervention.

Effect of Vitamin E and Selenium on Urine F_2-Isoprostanes, A Biomarker of Oxidative Stress

While vitamin E and selenium have antioxidant properties, few studies have investigated the effect of these nutrients on an oxidative stress biomarker, and none have investigated mediation of effects on a functional outcome such as lung function by an oxidative stress biomarker. This dissertation provides definitive evidence that vitamin E lowers a biomarker of oxidative stress and offers a stronger test of this hypothesis in comparison to previous small non-randomized studies reporting mixed results. Furthermore, the urine F_2-Isoprostane biomarker is considered the gold-standard for assessing *in vivo* oxidative stress, and many previous studies utilized biomarkers and specimen types that may reflect *ex vivo* processes. Selenium had no effect on F_2-Isoprostanes, consistent with its lack of effect on rate of decline in lung function(2). Lack of association between lowering of F_2-Isoprostanes and effect of vitamin E on rate of decline in FEV_1 may reflect the need for a more lung tissue-specific biomarker, as F_2-Isoprostanes are reflective of systemic lipid peroxidation.

The Response to Vitamin E Supplementation in the Respiratory Ancillary Study to SELECT

While vitamin E is posited to have beneficial health effects, and thus supplementation is suggested as a potential treatment or preventive agent, little is known

about the individual response to supplementation. Variability in plasma tocopherol levels, particularly the range observed in individuals consuming supplements(5) suggests that there is significant between-individual variability in plasma change in response to vitamin E supplementation. While several large randomized controlled trials of vitamin E showed no benefit on on health endpoints, achieved plasma vitamin E levels in response to supplementation were rarely considered. Recent studies have identified genetic variants that affect usual plasma vitamin E concentration(6) and response to vitamin E supplementation(7), however the data addressing plasma response is very limited. This dissertation addresses a gap in the literature by assessing associations between individual characteristics and plasma response to supplementation within a long-term, randomized control trial of a diverse sample including a large percentage of African Americans and cigarette smokers. Furthermore, use of the GC-MS methodology allowed for consideration of both alpha(α)-tocopherol and gamma(γ)-tocopherol; γ-tocopherol is increasingly under study in relation to bioactivity and health outcomes, but, as yet, very few papers are published on this topic. Since α-tocopherol and γ-tocopherol both use the same transfer protein (α-tocopherol transfer protein , α-TTP)(8), levels in the plasma are inversely correlated. Due to this inverse association, and considering that α-tocopherol supplementation causes a reduction in plasma γ-tocopherol levels, addressing plasma levels of α-tocopherol in comparison to γ-tocopherol is a more complete characterization of the effects of supplementation on plasma nutriture.

Race was identified as the most important predictor of change in plasma α-tocopherol and γ-tocopherol levels; in response to vitamin E supplementation over three years, African Americans had lower baseline α-tocopherol and less of an increase, and lower γ-tocopherol

with less of a decrease, compared to European Americans. This suggests that African Americans may benefit less from intervention, given that the effect of vitamin E on lung outcomes is proposed to act locally at target tissue and this nutrient must be carried in the plasma to the target tissue. It is unclear, however, whether the lower changes in response to supplementation indicates lower potential to benefit from intervention. A second possibility, which cannot be addressed by this dissertation, is that African Americans are using more of the supplemental vitamin E, and thus vitamin E is 'used up' leading to lower concentrations in plasma. If the latter scenario is correct, African Americans may actually have more potential to benefit from intervention; in the study of the oxidative stress biomarker, African Americans had higher levels of urine F_2-Isoprostanes, contributing some evidence to the plausibility of this hypothesis. Adjustment for cholesterol and other covariates, including smoking status and body mass index, indicate that the effect of race is robust and should be considered in future studies of vitamin E intervention. The Contribution of Baseline Nutriture To Intervention Effects in a Randomized Controlled Trial

Given that there was no effect of selenium on rate of decline in lung function in RAS(2), we undertook a plausibility analysis of selenium effects in men with lowest selenium levels at baseline, who were hypothesized to have the greatest potential to benefit from intervention. The null finding from the overall RAS(2) was thus further considered, and we tested whether treatment effects for selenium differed by baseline selenium nutriture, which was modeled as a continuous variable (ng/mL plasma concentration) and a categorical variable (deficiency, and percentiles). There was no evidence for an effect of selenium intervention on rate of decline in forced expiratory

volume in the first second(FEV_1)with consideration of baseline selenium levels. Additionally, there was no association between selenium nutriture at study baseline and rate of decline in lung function in this sample.

While it is evident that selenium has no effect on lung function in these men, it is important to note that this sample had a right-shifted distribution of baseline selenium in comparison to a prior study of selenium and lung function(3), and only 1.6% of men were selenium deficient according to a cut-point of selenium deficiency identified by a previous study of selenium supplementation and cancer(4). It remains a possibility, therefore, that selenium intervention may have a beneficial effect in men with lower selenium levels, but this hypothesis could not be fully assessed in this dataset given the overall high levels of selenium nutriture.

Significance and Future Directions

Vitamin E deserves further study as a potential treatment or preventive for oxidative stress-related health outcomes, including Chronic Obstructive Pulmonary Disease (COPD) given the suggested effects on lung function. There is no effect of selenium on an oxidative stress biomarker, nor on lung function, however assessment of effects in a sample with lower baseline selenium would enhance our understanding of associations between selenium and oxidative stress. Randomized controlled trials are the gold-standard study design, and offer the benefit of reducing the effect of confounding, particularly as trial size increases; however, analyses beyond intent-to-treat analyses add to our understanding of intervention effects and make full use of the large investment needed to produce such datasets. Future studies should assess baseline nutriture as well as the biokinetics of

plasma response to supplement interventions to gain a better understanding of effects on health endpoints.

References

1. Blumberg J., Heaney R.P., Huncharek M., et al. Evidence-based criteria in the nutritional context. Nutr.Rev.Nutrition Reviews 2010;68:478-84.

2. Cassano PA, Guertin KA, Kristal AR, et al. A Randomized Controlled Trial of Vitamin E and Selenium on Rate of Decline in Lung Function in preparation.

3. Hu G, Cassano PA. Antioxidant Nutrients and Pulmonary Function: The Third National Health and Nutrition Examination Survey (NHANES III). American Journal of Epidemiology 2000;151:975-81.

4. Duffield-Lillico AJ, Dalkin BL, Reid ME, et al. Selenium supplementation, baseline plasma selenium status and incidence of prostate cancer: an analysis of the complete treatment period of the Nutritional Prevention of Cancer Trial. BJU Int 2003;91:608-12.

5. Ford ES, Ajani UA, Mokdad AH. Brief Communication: The Prevalence of High Intake of Vitamin E from the Use of Supplements among U.S. Adults. Annals of Internal Medicine 2005;143:116-20.

6. Major JM, Yu K, Wheeler W, et al. Genome-wide association study identifies common variants associated with circulating vitamin E levels. Hum Mol Genet 2011;20:3876-83.

7. Major J.M., Yu K., Weinstein S.J., et al. Genome-wide association study identifies three common variants associated with serologic response to Vitamin E supplementation in men. Journal of Nutrition 2012;142:866-71.

8. Kelleher J, Losowsky MS. The absorption of α-tocopherol in man. British Journal of Nutrition 1970;24:1033-47.

CHAPTER 6

APPENDIX

Selection and Checking of the Mixed Model:

The Respiratory Ancillary Study (RAS) longitudinal dataset was limited to pulmonary function tests (PFTs) prior to March 1, 2009 to approximate the supplemented period. Supplementation ended on October 23, 2008 but we are conservatively allowing for the possibility of effects of supplement four months beyond supplement withdrawal. Standard quality control criteria, as detailed by the American Thoracic Society{{229 American 2005;}}, were applied to the dataset; tests not meeting start or end of test criteria or reproducibility were eliminated. The resulting dataset had 6,548 PFTs.

Different variance-covariance matrices were investigated, including unstructured, heterogeneous autoregressive, heterogeneous compound symmetry, heterogeneous toeplitz, variance components, autoregressive, and compound symmetry. A basic model including only elapsed time (months) to predict FEV1 was run under each variance-covariance matrix variation by altering the *type* option in the mixed model in SAS. AIC criteria and degrees of freedom were compared, and variance components was selected as the best choice for this longitudinal dataset given that it had a relatively small AIC value (92500.7) combined with the smallest number of degrees of freedom for the Likelihood Ratio Test (df=1). While some other choices, including unstructured, had smaller AIC values (unstructured, heterogeneous autoregressive, heterogeneous compound symmetry, heterogeneous toeplitz all had AIC of 92487.9), these models also had a greater number of degrees of freedom (df=3), thus variance components was a more parsimonius choice

given that it describes the structure just as well but with fewer parameters (since it assumes no correlation). Variance components models a different variance component for each random effect(1).

The Kenward-Rodgers method for standard error and denominator degrees-of-freedom (ddfm) correction was selected (ddfm=kr) for computing the denominator degrees of freedom for the tests of fixed effects; this method inflates the estimated variance-covariance matrix of the fixed and random effects and may shrink the standard error(2).

Previous experience with longitudinal pulmonary function datasets strongly suggested that reasonable rate of decline estimates could only be obtained with measures separated by a minimum of two years, and thus we eliminated additional pulmonary function tests (beyond the baseline PFT) from participants with less than two years from first to last PFT; baseline PFTs remained in the dataset for these participants, and thus they still contributed information to the model.

Residuals were studied using a basic mixed model, independent of the effect of treatment; the mixed model was used to predict rate of decline in FEV1, and time (elapsed months), age, height, and race were included as independent variables. Due to memory limitations in SAS related to the complexity of our longitudinal dataset, in order to output a dataset of residuals we were required to change the ddfm option to bw (between and within method), versus the Kenward Rogers method we had selected.

In comparing parameter estimates between the simple model using ddfm=bw and ddfm=kr, there were negligible differences in parameter estimates and thus use of

ddfm=bw to identify outliers is arguably sound. Studentized residuals were plotted against the predicted values (Figure 1), and no discernible pattern was detected; residual plots were comparable across treatment arms (data not shown).

Outliers, identified as observations (single pulmonary function tests) beyond a studentized residual value of ±3, were eliminated from the dataset. This process continued iteratively until no outliers remained. Once all outliers were identified and eliminated (116 outliers eliminated of the initial 6,548 observations), we again eliminated additional pulmonary function tests (beyond the baseline PFT) from participants with less than two years from first to last PFT to allow for more precise estimation of rate of decline (33 PFTs), resulting in a total of 6,397 PFTs. The effect of treatment was estimated in datasets that included and excluded outliers, and parameter estimates were comparable; the dataset excluding outliers more precisely estimated the effect of treatment, as evidenced by lower standard errors (data not shown).

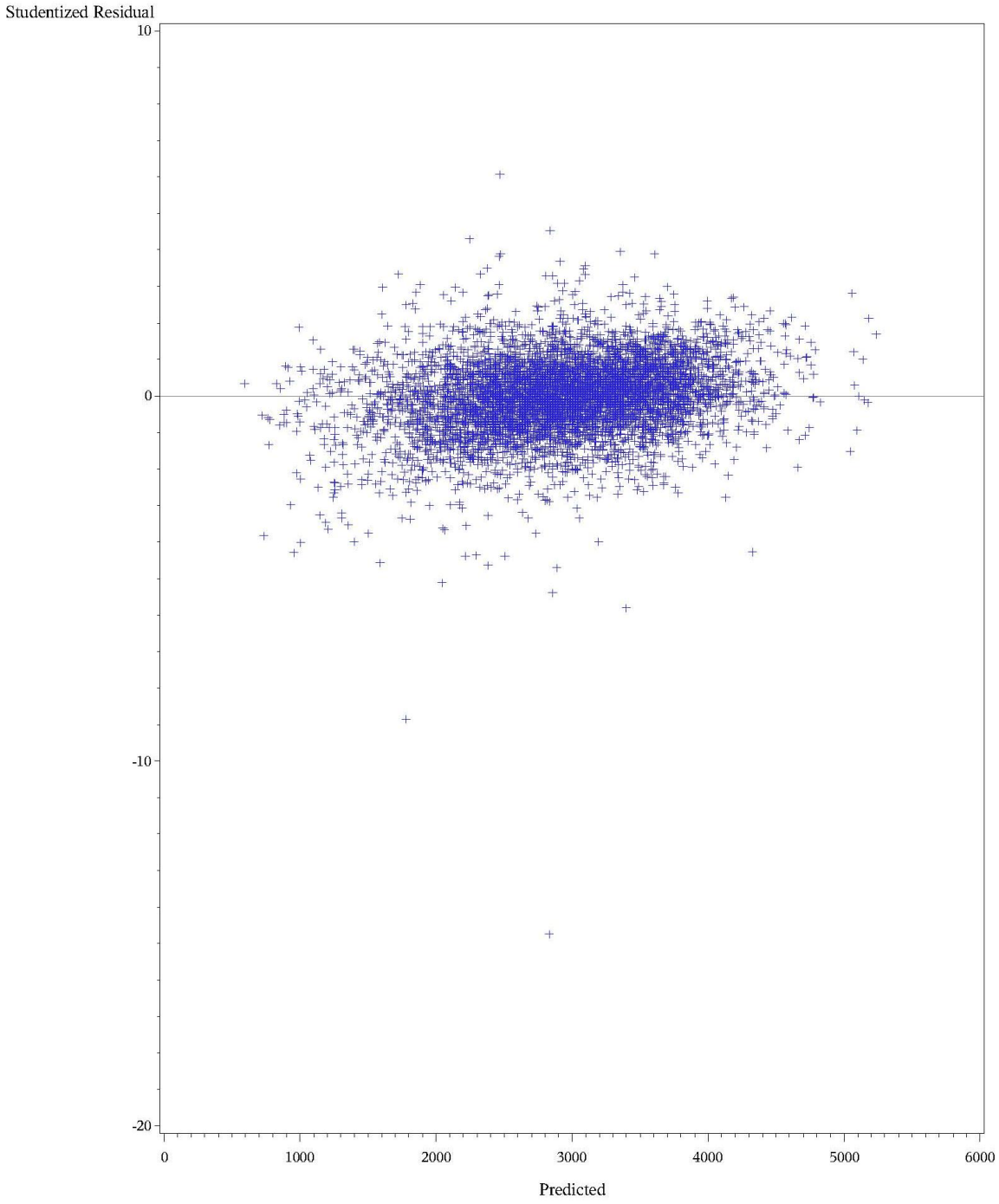

Figure 1. Studentized Residuals Compared to Predicted Values

References

1. SAS. SAS/STAT(R) 9.2 User's Guide, Second Edition: The Mixed Procedure. Internet: http://support.sas.com/documentation/cdl/en/statug/63033/HTML/default/viewer.htm#statug_mixed_sect018.htm#statug.mixed.mixedrandomtype (accessed January 04 2013).

2. SAS. SAS/STAT(R) 9.2 User's Guide, Second Edition: The Glimmix Procedure. Internet: http://support.sas.com/documentation/cdl/en/statug/63033/HTML/default/viewer.htm#statug_glimmix_a0000001411.htm#statug.glimmix.gmxmoddfm (accessed Jan 04 2013).